

A HISTOCHEMICAL STUDY OF THE DEVELOPMENT OF MEMBRANE BONE¹

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SIXTEEN FIGURES

INTRODUCTION

Of the several histochemical studies dealing with the development of bone, the most numerous of late have been those concerned with the incidence and distribution of alkaline phosphatase. The impetus set forth by Robison ('32) for more precise and detailed information in this field is not surprising and considerable evidence has accumulated correlating the presence of alkaline phosphatase in connection with the normal development of calcified tissues. Of several studies which have been concerned with this topic, Gomori ('43); Kabat and Furth ('41); Bourne ('43); Moog ('44); Bevelander and Johnson ('45, '49); Greep, Fischer and Morse ('48); Zorzoli ('48); Lorch ('47, '49a, b); Chang ('49); Follis ('49); and Cappellin ('47). We concur with the statement of Moog ('46) that they have only corroborated the well established fact, that phosphomonoesterase is an invariable concomitant of normal bone formation. Notwithstanding the evidence alluded to, Robison et al. were aware that phosphatase alone was not the only substance involved in calcification and accordingly postulated a so-called "second mechanism."

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A partial explanation of the second mechanism it appears, may well be related to the presence of glycogen in those areas undergoing calcification, according to the studies of Harris ('32); Glock ('40); Gendré ('38); Horowitz ('42) and Follis ('49b). Moreover, it would appear that identification of a phosphorylating enzyme in the epiphysis of growing bones adds another link in the chain of evidence. This presumes that glycogen in developing bones undergoes phosphorylation to produce a substance similar to glycerophosphoric acid utilized further as a substrate to supply phosphate ions (Gutman et al., '41, '42).

Roche ('46), however, maintains that while phosphatase is concerned with calcification, it serves merely as an activator or catalyst rather than as a causal agent in the splitting of phosphate ions. Furthermore, he emphasizes the possible role of the protein complex of the matrix in mineral fixation.

The identification and function of the chemical and morphological components of bone matrix comprises one of the large gaps in our knowledge of this tissue. Meyer ('38) isolated chondroitin-sulphuric acid from spongy bone, Dempsey et al. ('47) identified a mucopolysaccharide in cartilage matrix, and Follis and Berthrong ('49) observed metachromasia in the fibrillar structure of both cartilage and bone. In the matrix of dentin, Wislocki, Singer and Waldo ('48), and Engel ('48) have also investigated the presence of a polysaccharide in this calcified tissue.

The problem of mineralization in bone matrix is exceedingly complex and has been the subject of numerous chemical investigations. The studies of McLean and Bloom ('40); Bloom and Bloom ('40) indicate that the initial calcification of osseous tissue occurs simultaneously with the formation of the matrix. Bloom and Bloom ('40) also suggest that diverse physiological conditions and species differences may account for alterations in the histological picture.

PROBLEM

The obvious lack of detailed factual information bearing on the mechanism of bone formation need not be emphasized

at this juncture. The problem to be detailed in the subsequent paragraphs is concerned with a cytochemical study of the histogenesis of membrane bone. In this investigation we have utilized a number of modifications in technical methods. These permit a more accurate and detailed visualization of structural elements in connection with bone formation than has heretofore been reported. Further, it has been our purpose in this study to attempt to interrelate the chronological appearance and sequence of events existing in developing bone with reference to alkaline phosphatase, glycogen, ground substance and mineralization.

MATERIAL AND METHODS

The material used in this study consisted of embryo pig heads ranging in size from 28–40 mm. This stage of development permits sectioning without decalcification and also permits studies to be made on the whole sequence of bone formation through calcification.

For the identification of phosphatase some material was fixed in cold 95% alcohol, some in absolute alcohol at -40°C . and still others in an isopentane liquid nitrogen mixture at -170°C . Most blocks were cut without decalcification, a few were decalcified before cutting. Incubation solutions were used as described by Gomori ('39), and also a modification of this formula, in which magnesium chloride was substituted for calcium nitrate. The pH of these solutions was adjusted to 9.2. Incubation time varied from one-half to two hours.

Material to be used for the identification of glycogen and glycoprotein was fixed in 70% and 95% alcohol, and by the freeze-dry method. The methods of Gage and of Hotchkiss ('46) were utilized to identify glycogen; the latter method was also employed to localize polysaccharides after the sections had been digested with saliva.

For the visualization of mineral deposits, undecalcified material fixed by the freeze-dry method was used. Bloom and Bloom ('40) and Gersh's ('32) modification of the von Kossa method was employed.

OBSERVATIONS

Phosphatase. The histochemical localization and assignment of the possible role which alkaline phosphatase plays in the histogenesis of calcified tissues such as bones and teeth is beset with a number of difficulties. The Gomori method of visualization while having many advantages, has likewise certain disadvantages in a study of bone due to the overall blackening of cells and matrix. Moreover use of decalcifying methods followed by subsequent reactivation of enzyme activity has in our experience a number of serious drawbacks. Thirdly, the method of fixation² in an aqueous medium also results in a loss of enzyme, Doyle ('48), Wang and Grossman ('49).

In the present study we have prepared sections of developing bone by several different methods. A fairly typical example of the visualization of phosphatase obtained by following a decalcification technique described by Lorch ('47) is shown in figure 1. The periosteal tissue, osteoblasts and osteocytes show considerable amounts of phosphatase, while the calcified matrix appears relatively negative. In contrast to this, attention is directed to figure 2 which is a phosphatase preparation of an undecalcified section of bone. Examination of this figure shows a very intense blackening of periosteal fibers, osteocytes, osteoblasts and matrix. In fact the amount of blackening in this preparation frequently obscures structural details.

In an attempt to overcome the disadvantages of the method of visualizing phosphatase in bone, by the two commonly employed procedures, typical results of which have just been described, we have used two modifications of the phosphatase method described by Gomori ('39). First, the Altmann-Gersh freeze-dry method of fixation³ was employed to minimize

²In addition to variations in results following fixation attention is also directed to variations in results following other modifications in procedure, Newman et al., '50.

³We are indebted to Dr. A. C. Taylor for assistance in preparing the freeze-dry specimens.

the loss of enzyme in processing the tissues. Secondly, the usual composition of the incubating substrate was modified in such a way that magnesium chloride was substituted for calcium nitrate. The use of these two modifications in visualizing phosphatase in bone we believe, allows for a more precise method of controlling phosphate precipitation and subsequent visualization in developing bone than has heretofore been possible.

Figure 3, for example, shows the differential blackening which occurs in developing spicules of the membrane bone of the mandible. The upper areas of bone in this figure are relatively dark while in the lower spicules the blackening at this magnification is relatively absent. Figure 4, is an adjacent section from the same mandible stained by the von Kossa method showing the relative degree of mineralization in this tissue. A comparison of these two figures makes it evident that by the method described it is possible to obtain a differential darkening of developing bone spicules when localizing phosphatase and likewise by suitable methods as shown in figure 4 to correlate this difference with the state of mineralization the bone has undergone.

Turning now to a more detailed analysis of the spicules shown in figure 3, different portions of these structures are illustrated in figures 6, 7 and 8. Figure 6, under oil illustrates the upper portion of the darkened spicule referred to in figure 3, while figures 7 and 8 represent the middle and lower portions of these spicules respectively. Figure 6, shows the usual components one would ordinarily expect to exhibit the enzyme, and in addition phosphatase positive granular appearing structures in the matrix which we interpret to be osteogenic fibers. This portion of the spicule is unmineralized. The section illustrated in figure 7 shows the gradual loss of this blackening until eventually as shown in figures 8 and 9, the matrix is lacking in this feature completely, the osteocytes, however, are still strongly positive for phosphatase. The presence of phosphatase in the osteogenic fibers of the bone matrix obviously precedes mineralization, and

may also be correlated with other changes in the ground substance which will be described later.

Glycogen. Glycogen is observed in the embryonic undifferentiated tissues as shown in figure 10 and also in the differentiated periosteal tissue as shown in figure 5. With the advent of further development and more complete organization of the bone spicule, the mass of cells which surround the developing matrix become heavily laden with glycogen figure 11. Whether all of these cells eventually become osteoblasts is debatable, but undoubtedly many of them eventually do attain this state of differentiation. During the actual deposition of matrix, these differentiated osteoblasts appear to lose their glycogen content and this continues until just before mineralization, it then appears from our observations that there is a decided gain in the glycogen content of the osteoblasts.

When active growth and mineralization occur however, glycogen again appears in abundance in both the osteoblasts and osteocytes figure 12. Except for one phase in the development of the trabeculae, glycogen is abundant in periosteal tissues, osteoblasts and osteocytes.

Mucopolysaccharides. The localization of mucopolysaccharides in connection with the development and calcification of bone follows: Reference to figure 5, a low power photomicrograph of a section of developing mandible obtained from the same jaw as the sections illustrated in figures 3 and 4, shows the following: In a very young spicule, prior to calcification, a positive reaction for this substance is observed in the developing tissue. Reference to figure 13, a freeze-dry preparation of the terminal portion of a developing spicule shows that the osteogenic fibers which are being incorporated into the spicule take a stain indicating the presence of mucopolysaccharide. This is also true for the fibers observed in the marginal border of the spicule which may be interpreted as osteoid. In these two areas the fibers give a definite stain for the polysaccharide. As calcification occurs, the intensity of this reaction is enhanced as shown by examination of the

lower part of the section shown in figure 5 and by comparison with figure 4, the von Kossa preparation. In a still more advanced stage of bone development shown in figure 14, one can observe that the entire matrix now takes on a much more intense staining reaction, indicative of a higher concentration of the polysaccharide in the bone matrix than is evident in a younger spicule represented in figure 5. In a more detailed examination of the distribution of mucopolysaccharide in bone matrix illustrated in figures 11 and 12 one can observe the very intense reaction obtained in bone trabeculae which have undergone considerable calcification. Beyond the visualization shown in these photos, it is not possible to ascertain a discrete localization of this substance. Reference to figures 11 and 12 will show that the distribution of mucopolysaccharide in bone matrix appears as a diffuse but uniformly distributed substance. On the basis of our observations, it appears that the mucopolysaccharide may be located in both the cementing substance and the fibers. The nature of the optical properties of this material precludes accurate differentiation between fibers and cementing substance.

Mineralization. Among other desiderata relating to the development of bone we wish to consider further the appearance, deposition and localization of minerals. The histological methods employed for this visualization have already been referred to. We are aware that by using paraffin impregnation methods we may be dealing with sections showing some loss or even redistribution of minerals, but within limits, we believe that these preparations are fairly valid.

In the representative sections of the developing mandible illustrated in figure 4, growth and mineralization occurs progressively, an observation which has been made by many previous investigators. This progressive growth and mineralization is clearly indicated in the high magnification area of the terminal end of a spicule treated by the von Kossa method shown in figure 15. Examination of the extreme terminal portion of a growing spicule which includes a number of osteogenic fibers under oil is illustrated in figure 16. This

figure shows that in addition to the mineral deposition in the obviously organized part of the spicule, the osteogenic fibers are impregnated with a silver precipitate indicative of mineral deposition, even before they become incorporated into a spicule, and perhaps before the osteoblasts in this region are fully differentiated.

It appears therefore from our observations that mineralization occurs much earlier in the organization of a bone spicule than generally supposed. Mineralization slowly progresses concomitantly as the osseous tissue becomes elaborated and organized. This early deposition of mineral salts is a point usually unobserved in studies of this kind.

DISCUSSION

In the present investigation we have studied the development of membrane bone with reference to the localization of several different chemical constituents. It occurred to us that it would be of considerable interest to make a detailed cytochemical study of membrane bone first because such information at this time is much needed; and second, to compare these results with the several studies extant on endochondral bone.

At this juncture it seems appropriate to review briefly how phosphatase, glycogen, mucopolysaccharides and mineralization are interrelated in the development of this tissue.

Phosphatase is abundant in the embryonic mesenchymal tissue which gives rise to periosteal fibers. The osteoblasts also contain considerable amounts of the enzyme before the bone spicule is organized. As the osteogenic fibers become associated with osteoblasts and while the pre-osseous tissue takes on the appearance of a spicule, the fibers enclosed in this area are strongly phosphatase positive. As calcification of the spicule proceeds, the enzyme diminishes in the organic matrix but continues to be present in the periosteum, peripheral osteoblasts and osteocytes.

Glycogen is present in the periosteal tissues confined as far as we can see intercellularly and also occurs abundantly in active osteoblasts as well as in embedded osteocytes.

Mucopolysaccharide is present in the osteogenic fibers and appears in the matrix of bone before calcification occurs and more intensely after calcification has taken place.

Our studies on mineralization have not yielded facts not shown previously, but it was felt that a check on the state of mineralization was important in connection with the other constituents which were being studied.

In regard to phosphatase we wish to emphasize that this enzyme is present before calcification takes place, and in this respect our observations on membrane bone confirm those of Chang ('49) and Lorch ('49a), who made similar observations in regard to endochondral ossification. We further wish to emphasize the fact that phosphatase is present in the organic matrix of the developing spicules, and as mineralization occurs, this property diminishes. This feature has also been noted by Lorch ('47, '49a). This observation may substantiate to some degree Roche's ('46) hypothesis in which he assigns phosphatase the role of an accelerator or regulator of phosphate production.

The role of glycogen in endochondral ossification has been invoked in regard to the so-called second mechanism to explain calcification. In membrane bone glycogen is abundant as we have already stated. One might draw a comparison between the presence of glycogen in periosteal tissues, osteoblasts and osteocytes in developing membrane bone with that of glycogen in hypertrophic cartilage, but this analogy seems labored at this time.

Numerous references have been made concerning the probable role which the protein constituents of bone and other calcified tissues play in the process of calcification. Up until this time however, our knowledge concerning this topic is practically nil. Our own studies have confirmed the preliminary observations of Follis ('49), who suggested the presence of polysaccharide in long bone matrix. What this finding means at the moment is a question. It indicates that certain

substances are present in the organic constituent of bone not heretofore recognized. More study is needed to relate its presence to any dynamic aspect of bone formation.

Our studies have shown that phosphatase and glycogen appear to be fairly constant constituents of the periosteum and bone cells during the development of membrane bone, the former diminishes as calcification proceeds. Mucopolysaccharide appears in the osteogenic tissue and is enhanced as mineralization occurs. It would appear from our studies that the histogenesis of bone is much more complex than one might be led to assume on the basis of previous explanations. While we have confined ourselves in this study to the identification of only 4 different constituents which occur during the histogenesis of bone, the recent studies of Cappellin ('48) and Follis ('49), strongly suggest that several other chemical constituents including diverse enzyme systems may also be involved in the elaboration of bone. The clarification of this problem awaits further studies along lines of investigation just indicated.

SUMMARY

1. A cytochemical study of developing membrane bone has been described with reference to the localization of alkaline phosphatase, glycogen, mucopolysaccharides and mineralization.

2. Phosphatase occurs in the embryonic mesenchyme, in the osteoblasts, osteocytes and in the young spicule of the fibrous matrix preceding calcification. As mineralization occurs, the enzyme becomes much reduced in the matrix, but is still found in the osteoblasts and osteocytes.

3. Glycogen is present in fibroblasts of the periosteal tissues, and also in osteocytes and osteoblasts during all phases of bone formation studied.

4. Mucopolysaccharide is likewise present in the fibers and fibroblasts of the periosteal tissues and later is found in the bone matrix. It appears more intense as mineralization occurs.

5. Mineralization appears to take place to some extent very early in the formation of bone, even before the spicule is well organized.

6. The occurrence of the constituents identified in the present study have been briefly discussed in terms of their possible significance to the problem of calcification.

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PLATE 1

EXPLANATION OF FIGURES

1 Section of membrane bone showing localization of phosphatase following decalcification and reactivation. $\times 600$.

2 Section of undecalcified membrane bone prepared according to the method of Gomori. Note intense blackening of structures. $\times 600$.

3 Freeze-dry preparation of the developing pig mandible treated to show the localization of alkaline phosphatase. Section was incubated in the modified magnesium substituted substrate mixture. The darkened areas indicate phosphatase. $\times 150$.

4 Section adjacent to the one shown in figure 3. Treated by the von Kossa method to show differential mineralization in the growing bone. The darkened areas in bone indicate the presence of mineral salts. $\times 150$.

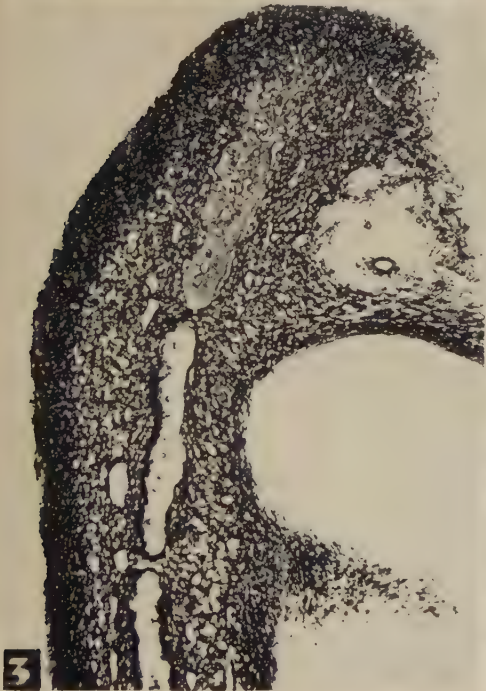
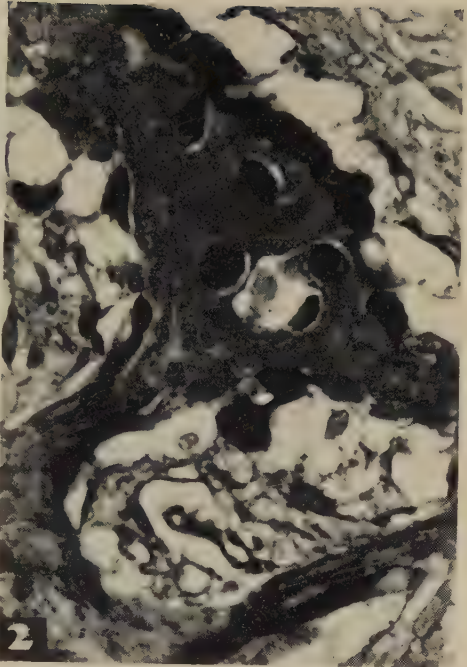
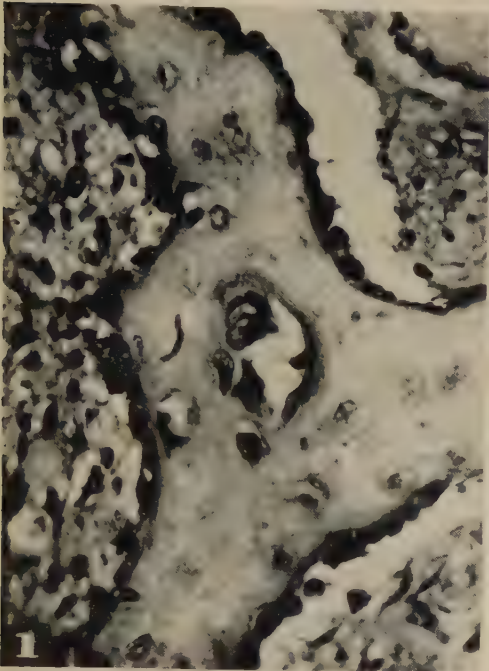


PLATE 2

EXPLANATION OF FIGURES

5 Freeze-dry preparation of section of mandible treated to show distribution of glycogen and mucopolysaccharide. The portion of the bone at the top of the photo is least mineralized (see fig. 4). $\times 150$.

6 Freeze-dry preparation of mandible. High power of upper portion of section shown in figure 3. Note phosphatase in bone matrix. $\times 1200$.

7 Middle portion of trabecula shown in figure 3. Note transition zone in matrix where phosphatase begins to disappear as mineralization takes place. $\times 600$.

8 Lower part of trabecula shown in figure 3. Note that in this portion of the trabecula that except for the osteocytes, phosphatase is relatively absent in the matrix. The bone shown in this photograph is mineralized. $\times 600$.

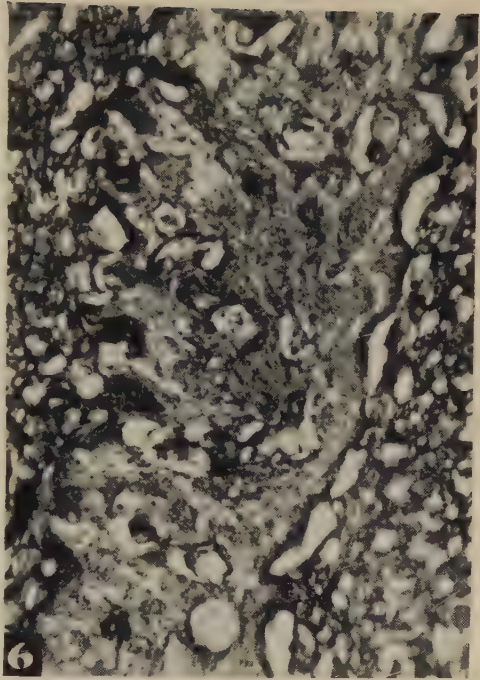
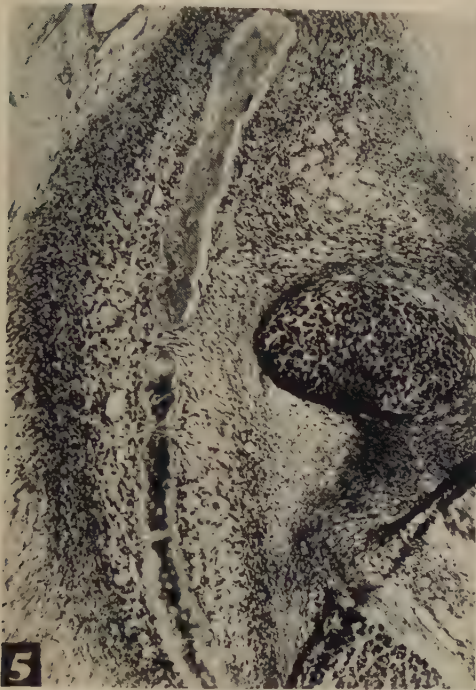


PLATE 3

EXPLANATION OF FIGURES

9 Freeze-dry preparation of mineralized portion of trabecula treated to show presence of phosphatase using magnesium modification of incubating solution. Note relative absence of the enzyme in the matrix proper. Osteocytes and their processes contain an abundance of the enzyme. $\times 1200$.

10 Uncalcified spicule fixed by the freeze-dry method and stained by the Hotchkiss method. The matrix shows presence of the polysaccharide and the young osteoblasts are heavily laden with glycogen. $\times 600$.

11. Partially calcified trabecula and surrounding periosteal tissue. Freeze-dry fixation. Hotchkiss stain. Note polysaccharide in the bone matrix and glycogen in periosteal tissues, osteoblasts and osteocytes. $\times 600$.

12 High power photo of portion of bone shown in figure 11. Note the diffuse character of the distribution of polysaccharide in the matrix and also the intensely laden cytoplasmic portions of osteocytes. $\times 1200$.



PLATE 4

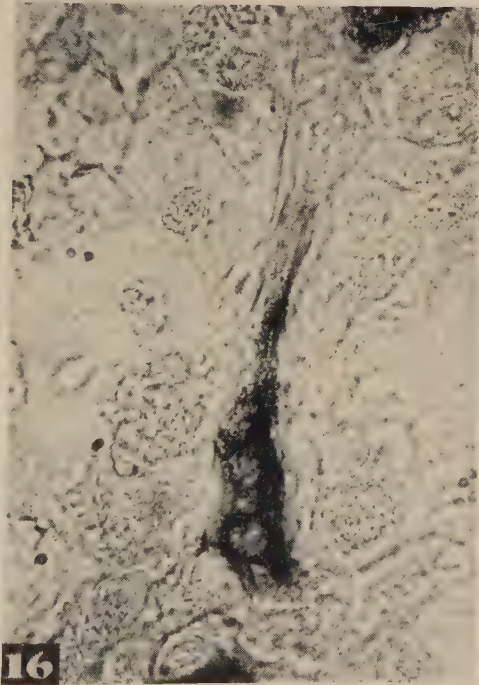
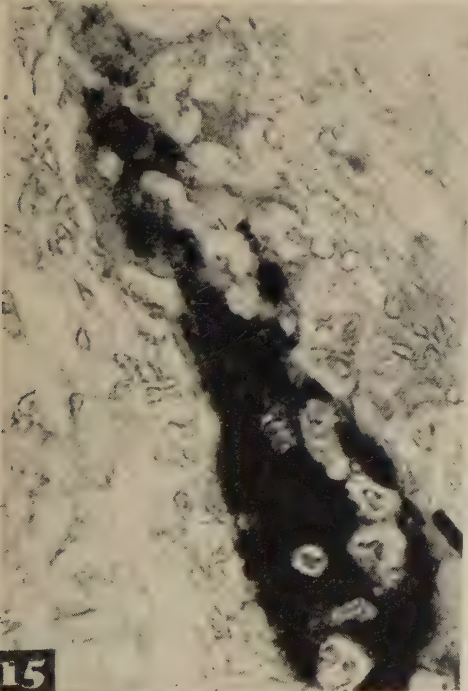
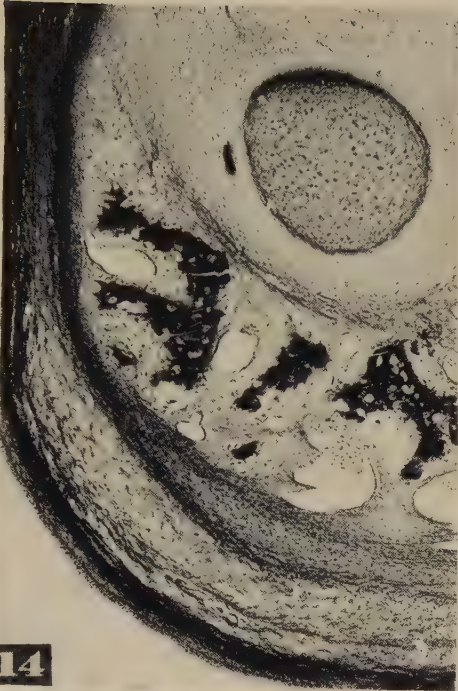
EXPLANATION OF FIGURES

13 Terminal end of a developing spicule showing presence of mucopolysaccharide in osteogenic fibers. Freeze-dry fixation, Hotchkiss stain. $\times 1200$.

14 Section of jaw showing a number of bone areas. Note fairly intense localization of mucopolysaccharide in calcified areas. Fixation 95% alcohol, Hotchkiss stain, subsequent digestion with saliva. $\times 150$.

15 Developing spicule showing presence of mineral salts. Freeze-dry fixation, von Kossa staining. $\times 1200$.

16 Preparation similar to that shown in figure 15. Note minute mineral deposition on fibers not yet incorporated into the spicule proper. $\times 1200$.



NEUROMUSCULAR SPINDLES IN THE EXTRAOCULAR MUSCLES IN MAN¹

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FOUR FIGURES

!

In the course of an earlier investigation by one of us (S. S., '46) on the pupilloconstrictor pathway in the human oculomotor nerve, structures possessing the characteristic morphological features of muscle spindles were repeatedly observed in all the extraocular muscles. In view of the generally expressed belief that eye muscles have no proprioceptive innervation it was decided to obtain more exact information about the total number and distribution of the spindles. A preliminary report on the findings of this enquiry has already been given (Sunderland, '49).

These structures were also observed by Daniel in '46 who later, in collaboration with Cooper, submitted them to a detailed examination which culminated in the publication of a paper "Muscle Spindles in Human Extrinsic Eye Muscles" ('49). This excellent account in which the literature is comprehensively reviewed, the morphological appearance of the spindles clearly described and convincingly illustrated, and their distribution and number in the inferior rectus recorded, covers essentially the same ground as that surveyed by us. Though this eliminates the necessity for a further detailed account it is felt that certain features of our enquiry are worth recording.

In Cooper and Daniel's paper precise information concerning the distribution and number of the spindles was confined to one specimen of the inferior rectus muscle. Detailed counts

¹ This work was assisted by a grant from the National Health and Medical Research Council of Australia.

were not made of the spindles in the other ocular muscles examined by them though the numbers were roughly estimated. We have obtained the number of the spindles and their distribution in 6 specimens of the superior oblique muscle and in one specimen each of the superior rectus, medial rectus and levator palpebrae superioris muscles. It is perhaps fortunate that the muscles selected by us for intensive study were those which were not examined in such detail by Cooper and Daniel as the inferior rectus on which their precise distribution studies were made.

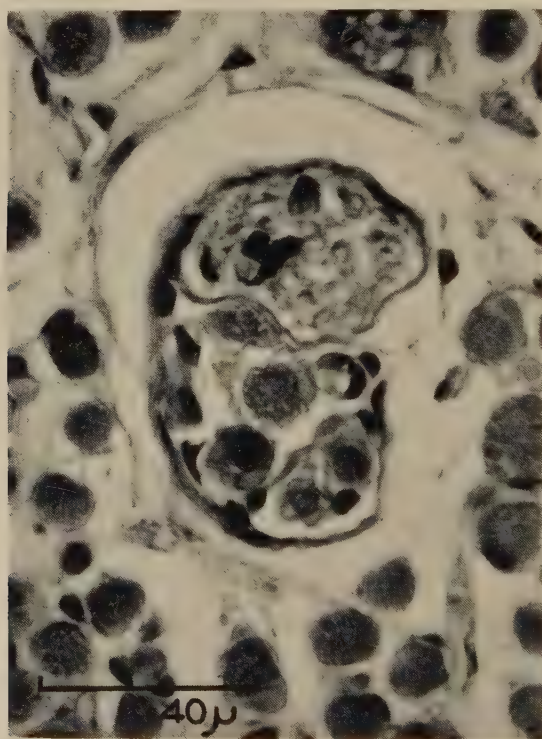


Figure 1

Figs. 1 and 2 Photomicrographs of transverse sections of specimens of the superior oblique (1) and superior rectus (2) muscles to illustrate morphological features of the spindle.

MATERIAL AND METHODS

The muscles were carefully removed from post mortem subjects within 24 hours of death, were gently stretched on card and fixed in 10% formol saline. Each muscle was dehydrated in the usual manner and embedded in paraffin. Six specimens of the superior oblique and one each of the superior rectus, medial rectus and levator palpebrae superioris muscles were cut transversely into blocks from which serial sections of the entire muscle were cut at 10μ . Every 10th pair of sections was mounted and one stained with haematoxylin and eosin and the other with Van Gieson's mixture. The interval between sections examined and charted for reconstruction was such that spindles less than 100μ in length, and in some speci-

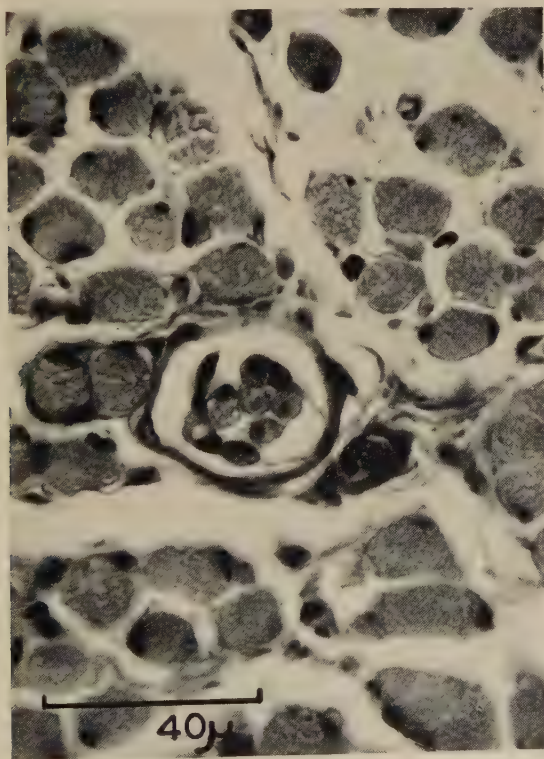


Figure 2

mens less than $300\ \mu$, may have been missed. Longitudinal serial sections of 4 specimens of the superior oblique and one specimen of the lateral rectus were cut at $10\ \mu$ and stained in the same manner. Numerous other specimens provided longitudinal and transverse sections which were prepared from areas ascertained to be rich in spindles. These sections were stained by a variety of silver methods as well as with the stains referred to above. This combined material proved suitable for an enumeration and distribution study of the spindles and an examination of their morphological features.

OBSERVATIONS AND DISCUSSION

The information provided by the investigation may be briefly stated:

1. It is confirmed that structures presenting the characteristic morphological features of neuromuscular spindles are obvious and constant features of all the human extraocular muscles (figs. 1 and 2).

2. In Cooper and Daniel's material the spindles were found in the proximal and distal thirds of the muscles and were absent from approximately the middle third. This was generally so in our material. In some specimens, however, spindles were found in the middle third (see fig. 3), the area in which they were absent being confined to an irregular zone which was placed more distally towards the junction of the mid and distal thirds of the muscle.

3. The central portion of the muscles which was free of spindles was the area devoted to motor end plates.

4. The number of spindles in the various muscles examined by us is given in table 1. The figures given by Cooper and Daniel for their specimen of an inferior rectus muscle are included for reference.

5. On their detailed count of the spindles in the inferior rectus and on their estimates in other muscles Cooper and Daniel concluded that "The inferior recti oculi always appeared to contain the greatest number of spindles . . . The obliques seemed to have a rather smaller complement than the

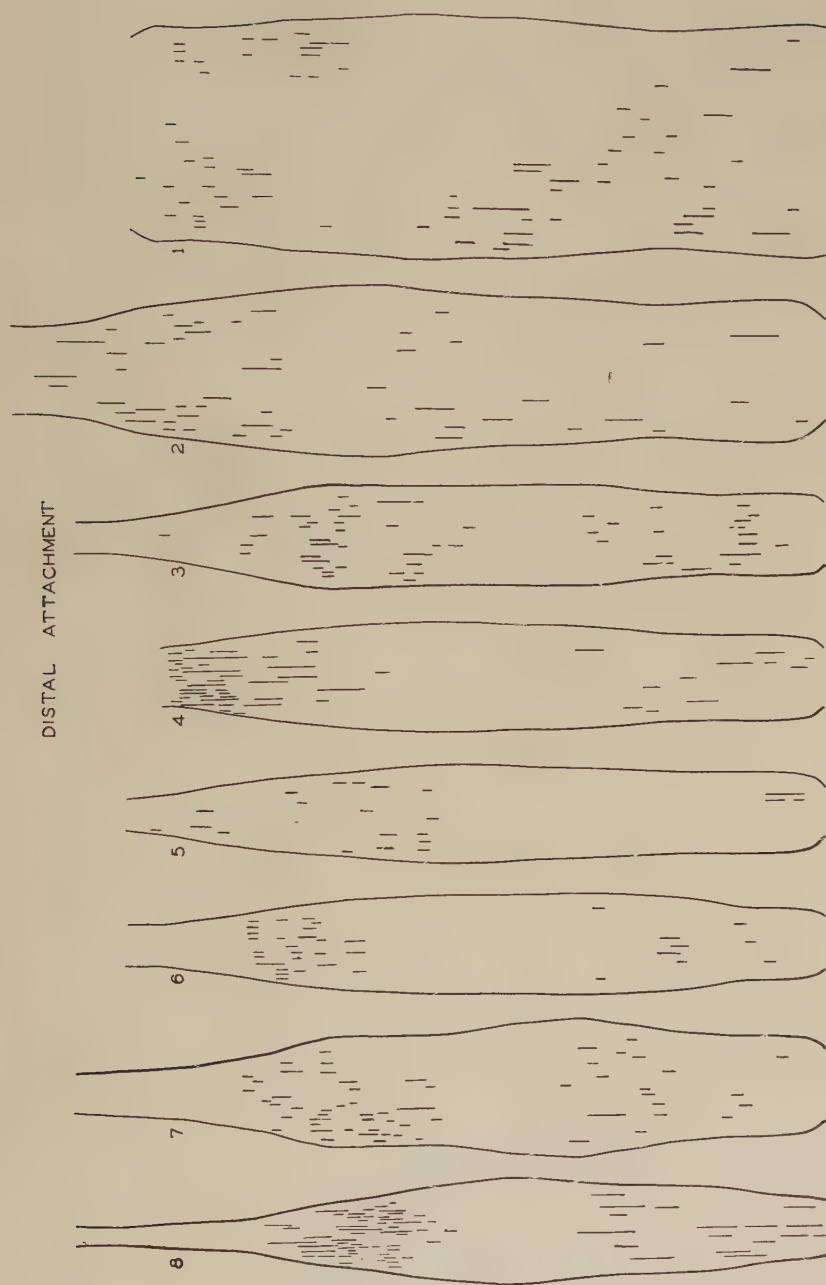


Fig. 3 Outline diagram of the medial rectus (1), the superior rectus (2) and superior oblique (3 to 8) muscles to illustrate the distribution and relative lengths of all the spindles, projected into one plane, in those muscles. Not to accurate scale.

recti . . . The levator palpebrae superioris is always poorly supplied with spindles." The levator palpebrae was poorly supplied in our material but the counts for the superior and medial recti and the superior oblique muscles indicated that all were equally richly supplied.

6. In Cooper and Daniel's specimen of the inferior rectus 41 spindles were described as being located in the proximal third and 6 in the distal third. The distribution of the spindles in our specimens is shown in table 1 and figure 3. With one exception the distal third of the muscle was more heavily studded with spindles.

TABLE 1

Number and distribution of neuromuscular spindles in the extraocular muscles

SUBJECT	SUPERIOR OBLIQUE		SUPERIOR RECTUS		MEDIAL RECTUS		COOPER AND DANIEL INFERIOR RECTUS	
	Proximal third	Distal third	Proximal third	Distal third	Proximal third	Distal third	Proximal third	Distal third
1	17	54	18	33	37	34	41	6
2	19	40						
3	11	40						
4	3	19						
5	9	26						
6	18	32						

7. The delicacy of the capsule of the spindle together with the details relating to the morphological features of the encapsulated intrafusal fibers and periaxial space were, in general, confirmed by us. Minor points on which our findings disagree with those of Cooper and Daniel are given in table 2.

8. We encountered the same difficulty as that experienced by Cooper and Daniel in reconstructing the details of the innervation of the spindle. Silver stains revealed a pattern of considerable complexity which requires a more detailed investigation than our material permitted.

9. The variation in the diameter of the extrafusal fibers reported by Cooper and Daniel was also observed by us. Dif-

TABLE 2

Morphological features of the neuromuscular spindles in the extraocular muscles in man

MORPHOLOGICAL FEATURE	COOPER AND DANIEL	MERRILLEES, SUNDERLAND AND HAYHOW
Length	50 μ to 1640 μ . Many about 500 μ and few (5 in the inferior rectus) longer than 1 mm.	100–200 μ to 2600 μ . Average approximately 900 μ . Approximately 30% of the spindles exceeded 1 mm in length in all specimens, while specimens of the superior oblique and medial rectus each contained one spindle exceeding 2 mm in length (see fig. 4).
Diameter	20 μ to 100 μ . Majority 40 to 80 μ .	20 to 100 μ . The majority 35 to 65 μ .
Number of intrafusal fibers	1 to 10. Usual number 2, 3, or 4.	1 to 15. Rarely more than 7 and less than 3. Average 4.
Diameter of intrafusal fibers	7 to 20 μ	5 to 30 μ with an average of 10 μ .

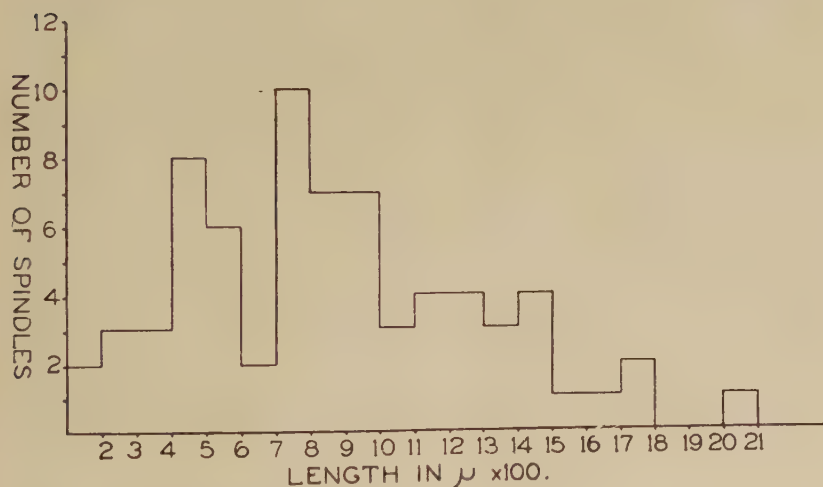


Fig. 4 Histogram illustrating the number of spindles of different lengths in a specimen of the superior oblique muscle.

ferences observed in the caliber of different fibers in transverse sections of muscles can only be accepted as evidence of variations in muscle fiber caliber if the fibers are arranged in parallel and if they maintain a constant diameter for the full length of the muscle. We are, however, subjecting this feature to further investigation in view of evidence in our material that many fibers do not extend the full length of the muscle and that they show tapering at the extremities and a swelling at the site of the motor end plate so that the transverse dimensions of the fiber are influenced by the level at which it is sectioned.

10. The implications of these findings have been fully discussed by Cooper and Daniel and call for no further comment by us.

SUMMARY

It is confirmed that structures presenting the characteristic morphological features of neuromuscular spindles are obvious and constant features of all the human extraocular muscles.

The number of spindles was counted and their distribution charted in 6 specimens of the superior oblique muscle and in one specimen each of the superior and medial recti muscles. The number varied from 22 to 71. The spindles were confined to the proximal and distal thirds of the muscle and were absent from approximately the central third. With one exception the distal third was more heavily studded with spindles.

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CLEAVAGE OF UNFERTILIZED OVA IN IMMATURE FERRETS

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EIGHTEEN FIGURES

The fate of unfertilized ova has been studied in the mouse (Charlton, '17), the rat (Mann, '24) and the rabbit (Pincus, '30). Recently the fragmentation of unfertilized rat ova (which closely resemble the segmentation of fertilized ova) has been further described by Austin ('49). Of Mustilidae, the cleavage of fertilized ova has been studied in the ferret (Robinson, '18), the mink (Enders, '38; Hannson, '47) and the long-tailed weasel (Wright, '46), but no detailed report on the behavior of unfertilized ova in these species has been published. This paper reports observations on unfertilized ferret ova, their cleavage, and their nuclear configurations.

ANIMALS AND METHODS

The animals were young ferrets born in this laboratory at the end of March or the beginning of April 1949, as a result of the light treatment of their parents.¹ They were injected with Pregnant Mare's Serum (PMS)² in the middle of August. In general, the method followed was that suggested by Rowlands (personal communication). Four or 5 subcutaneous injections (40 to 60 I.U. each) were given at an interval of two to 4 days, depending on the vulvar swelling, in order to cause the growth of follicles. A final subcutaneous injection of 200 I.U. of the same preparation was given in order to

¹ Work by J. Hammond, Jr.

² Antex from Ayerst, McKenna and Harrison Co.

induce ovulation. The animals were killed from 32 to 120 hours after final injection. The uterus and the ovarian capsule were first separated. The uterus was flushed with saline by means of a broad pipette and the ovarian capsule with attached tube was placed in a small watch glass containing saline. Under a stereoscopic microscope, the tube was dissected from the ovary and then flushed with saline (using a very fine capillary pipette according to the technique of Hammond ['49] on mouse ova). After recovery, the ova were photographed and some of them were fixed in a clot of serum with Bouin's solution. They were sectioned at 10μ and stained with hematoxylin. A few representative ovaries (one with tube) were also sectioned and stained.

RESULTS

Eleven animals were used. No stimulation of follicular growth was observed in two very young animals, luteinization of follicles occurred in one and no ovum was recovered from a 4th animal. All together 41 ova were recovered from the remaining 7 animals. Table 1 summarizes the condition of the ova and the site of their recovery. They can be classified as ova with closely attached corona radiata cells, with loosely attached corona cells, and as naked ova. This classification may provide an indication of their age, since the denudation of unfertilized rabbit ova takes a definite length of time after ovulation (Pincus, '30).

Two ova (fig. 1) were recovered from the capsule of ferret no. 1 during the dissection of the tube from the ovary. They were very recently ovulated. The cytoplasm of the ova was evenly dark and without the large yolk granules that may be seen, for instance, in the cow ovum. The corona cells were thick and had very long, fine processes which apparently penetrated the zona pellucida. These cells formed three layers surrounding the ovum; a light inner layer with thread-like processes, a dark middle cellular layer and a light outer layer with rather sharp, fine processes (fig. 1).

About half of the ova recovered were still closely surrounded with corona cells, though the cells of the outer layer were smooth and round (figs. 2 and 3). Twelve out of 17 ova in this group had cleaved fairly equally into two to 6 cells (figs. 2, 3 and 4) and appeared to be very similar to the segmenting fertilized ova.

TABLE 1
Cleavage of unfertilized ferret ova

HOURS BETWEEN FINAL IN- JECTION AND EXAMINA- TION	FER- RET NO.	TOTAL	OVA RECOVERED					REMARKS
			Cleav- age or frag- menta- tion	From tubes			From uteri, naked degen- erated	
				Corona cells tightly at- tached	Corona cells loosely at- tached	Naked		
32-34	1	7	2 ¹	2 ³	3	2	0	No ovum found from right tract
	2	11	3 ¹	1	3	4	3	
48	3	1	0	1				No ovum found from left tract
71-72	4	9	6 ²	9				Right tube sectioned
	5	2	1 ²	2				
96	6	8	4 ²	7			1	
120	7	3	1 ²	1		1 ²	1	
Total	7	41	17	23	6	7	5	

¹ Unequally cleaved and fragmented.

² Equally cleaved 2 to 6 cells.

³ Recovered from capsule.

A few ova (6 out of 41) were either very loosely surrounded with corona cells or in the cumulus cell mass (figs. 5 and 6). In this group, most of the ova were fragmented into two unequal cells or had cytoplasmic extrusions, which were obviously degenerating.

There were 12 naked ova (figs. 6, 7 and 8). They were very dark and most of them had degenerated. Only one of them had cleaved fairly evenly into 4 or 5 cells with some fragments (fig. 7). The 5 naked ova recovered from the uterus

were definitely degenerated; two of them were very dark and three very light (fig. 8).

In ferrets nos. 1 and 2, freshly ovulated ova, naked, and uterine ova were recovered at the same time. This indicates that ovulation had occurred following both the low and high dosage of PMS. Thus the age of the ova could not be accurately estimated. Furthermore, the time at which ovulation occurs following the injection has not been determined. However, it is quite possible that most of the ova were ovulated 30 hours after injection as they are after copulation (Hammond and Walton, '34). If this is so, the denudation of ova may be estimated to occur at about 48 hours (ferrets no. 1 and no. 2 which had ovulated following the last low dose of treatment) or 66 hours (ferret no. 6 which ovulated following the final high dose of treatment) after ovulation.

The size of fresh ova with attached corona cells was found to be 240–300 μ ; the naked ova measured 140–150 μ and the zona pellucida, 15–20 μ .

Eleven ova were fixed. After sectioning and staining only 8 were analyzable. One ovum from ferret no. 4 and three from ferret no. 6 were uncleaved, but two of these ova (from no. 6) had several cytoplasmic extrusions.

Of the uncleaved ova, the first, recovered from ferret no. 4, had the first polar body and a group of chromosomes (fig. 9). The chromosomes were clumped together and there was no obvious spindle. The chromosomes were probably degenerating after the formation of the second maturation spindle. The number was 16 or 18 (i.e., haploid, according to Koller, '37). No nucleus was observed in the second ovum but it had a large polar body in the process of cleaving. Nine scattered dark dots were present in the polar body. The third had two nuclei near each other in the center of the ovum, one large and one small, both with a clear nuclear membrane. The 4th one (fig. 10) had 9 small nuclei with clear nuclear membranes. They were scattered in the periphery of different sections.

Of the 4 fairly evenly cleaved ova, the first recovered from ferret no. 4 had three cells, two of which had large, deeply

stained nuclei. The second ovum had 6 cells and a polar body; 4 of which had fairly large irregular nuclei (figs. 11 and 12). The third ovum had three cells; one cell had two large nuclei (fig. 13); the other two cells had one or two nuclei in the periphery (fig. 14). The 4th ovum had 5 cells with a polar body among them; two cells each had spindles and contained 16 or 17 chromosomes (figs. 15 and 16).

The right tube of ferret no. 6 was sectioned in order to study the condition of the ova and their location. Sixteen ova, all with corona cells attached, were observed in the 6th and 7th loop of the tubes which is about halfway to the uterus. The "cleavage" of the ova (fig. 17) and their nuclear configurations were in general not different from the 8 ova just described, with the exception that the nuclei of two cells appeared to be normal (fig. 18). It is interesting to note that the ova are transported *en masse*. It is evident from the number of ova ovulated in ferret no. 6 (all together 24) that treatment with PMS may induce superovulation.

DISCUSSION

The fragmentation or apparent parthenogenesis of ova in the atretic follicles is a common phenomenon (Courrier, '23; Engle, '27), but "cleavage" of unfertilized ova in the tube has been considered to be rare. In the rat, Mann ('24) has reported 9 cleaved (two to 4 cells) out of 252 ova examined. Recently Austin ('49) has reported, and beautifully photographed, the occurrence of 14 to 20% "cleaved" rat ova. In the ferret, the occurrence of such cleavage is still higher (12 out of 41) as revealed in the present study. In the mink, the author has observed one evenly cleaved three-cell ovum out of 15 unfertilized ova examined. In the rabbit, the fragmentation or cleavage of unfertilized ova one day after an ovulation-inducing injection is very rare. However, when rabbits are examined two days after the ovulation-inducing injection, 20-30% fragmentation or "cleavage" was observed by the author in two rabbits out of about 30. Nevertheless, the "cleavage" is quite different from that of normal fertilized ova;

the number of cells was lower than expected in a fertilized ovum; the cells were compact and were unevenly transparent and irregular.

The nuclear configurations of the "cleaved" ferret ova in general are similar to the descriptions of fragmented ova in atretic follicles although no morula form was observed as reported by Courrier ('23). One peculiar feature observed in the present study was the presence of a spindle in each of two separate cells of an ovum. But whether all the nuclei in the cells had arisen as a result of spindle formation or simply from scattered chromatin material is not known.

It is interesting to note that all the "cleaved" ferret ova were still surrounded with corona cells. If the corona cells supply nutriment to the ovum, the high incidence of "cleavage" rather than immediate degeneration in the ferret ova may be due to adequate nutrition. Denudation of unfertilized rabbit ova occurs 6 to 10 hours after ovulation (Pincus, '30) and it probably takes two to three days in the ferret, hence the higher incidence of cleavage in the ferret may be due to this prolonged adherence of the corona cells. This assumption also explains the high percentage of regular cleavage (76.4) in the young unfertilized rabbit ova (with corona cells) and the low percentage (27.7) in the old ova (naked) as reported by Pincus ('36).

In an attempt to fertilize rabbit ova *in vitro*, the author occasionally observed that about 50% of unfertilized ova cleaved in culture where no spermatozoa were added. Several nuclei in one cell or the presence of nuclei of different sizes in two or three cells were often observed. It seems probable that the "cleavage" of unfertilized ova in atretic follicles, in the tubes or in culture is a manifestation of abnormal cytoplasmic and nuclear activity under favorable conditions before degeneration.

The cleavage of unfertilized ferret ova cannot be ascribed entirely to the PMS treatment or the immaturity of the animals because the occurrence of "cleaved" but unfertilized ova in various groups of rats is not extremely different (Austin, '49).

SUMMARY

Eleven immature ferrets were subjected to treatment with Pregnant Mare's Serum to induce the growth of follicles and ovulation. Forty-one unfertilized tubal and uterine ova were recovered from 7 animals. Denudation of the ova was estimated to occur about 48 or 66 hours after ovulation. Twelve ova were fairly equally "cleaved" into two to 6 cells appearing very similar to segmenting fertilized ova. Most of the cells in the "cleaved" ova had one nucleus or two nuclei of different sizes and appearances, but a spindle with a haploid number of chromosomes was observed. The "cleavage" of unfertilized ova is perhaps a manifestation of abnormal cytoplasmic or nuclear activity under favorable conditions before degeneration.

The author wishes to acknowledge his sincere thanks to Dr. G. Pincus for criticisms and to Miss Jacqueline Choiniere for the histological preparations. This work has been supported by grants from the Committee of Human Reproduction, National Research Council, acting on behalf of the National Committee on Maternal Health and from Mr. Benjamin Harrison Weiss.

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PLATE 1

EXPLANATION OF FIGURES

Photomicrographs of fresh unfertilized ferret ova. All photographed with 16 mm objective, $\times 72$, except figure 7 which was photographed with 4 mm objective, $\times 317$.

1 A very recently ovulated ovum, slightly pressed, recovered from ovarian capsule of ferret no. 1.

2 An ovum cleaved into two cells with closely attached corona cells, recovered from ferret no. 5.

3 and 4 Ova cleaved into 4 to 6 cells recovered from ferret no. 6.

5 An ovum fragmented into two unequal cells with corona cells and cumulus mass loosely attached, recovered from ferret no. 1.

6 Four ova recovered from ferret no. 2. Two ova with corona cells loosely attached, three of them fragmented into two unequal cells.

7 One naked ovum of ferret no. 7, cleaved into 4 or 5 cells with fragments.

8 Two naked ova recovered from the uterus of ferret no. 2.

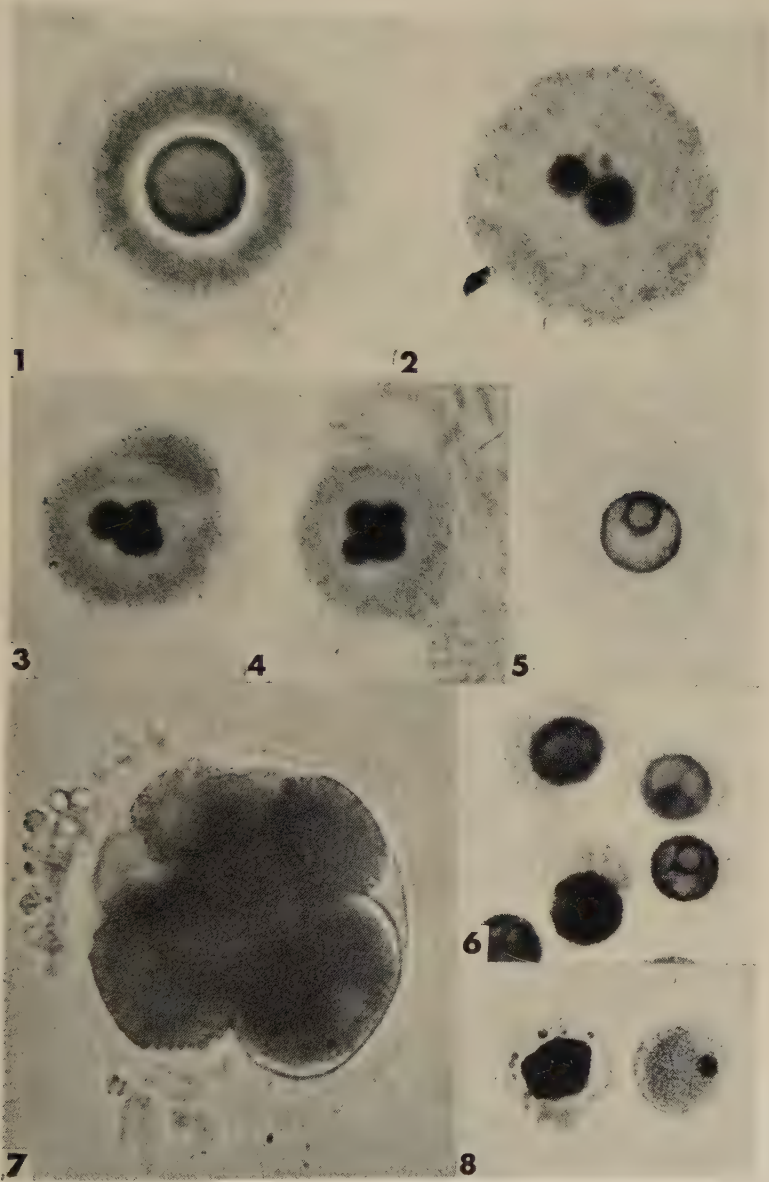


PLATE 2

EXPLANATION OF FIGURES

Photomicrographs of sections of unfertilized ferret ova. All photographed with 4 mm objective. $\times 255$.

9 One section of an ovum with chromosomes clumped together and its chromosomes photographed with oil immersion (inset).

10 One section of an ovum showing three small nuclei.

11 and 12 Two sections of a 6-celled ovum. Irregularity of nuclear materials.

13 and 14 Two sections of a three-celled ovum. The nuclei of two cells are photographed with oil immersion (inset).

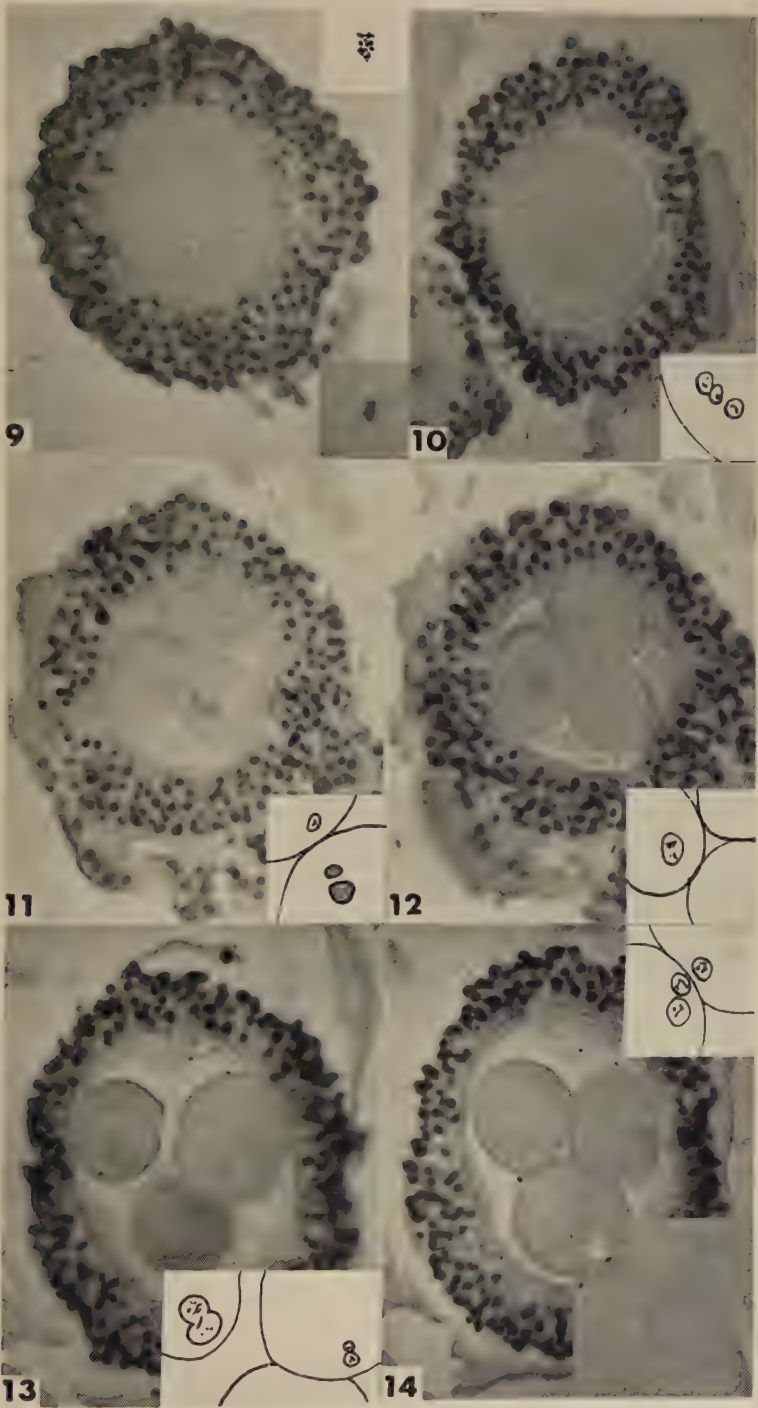


PLATE 3

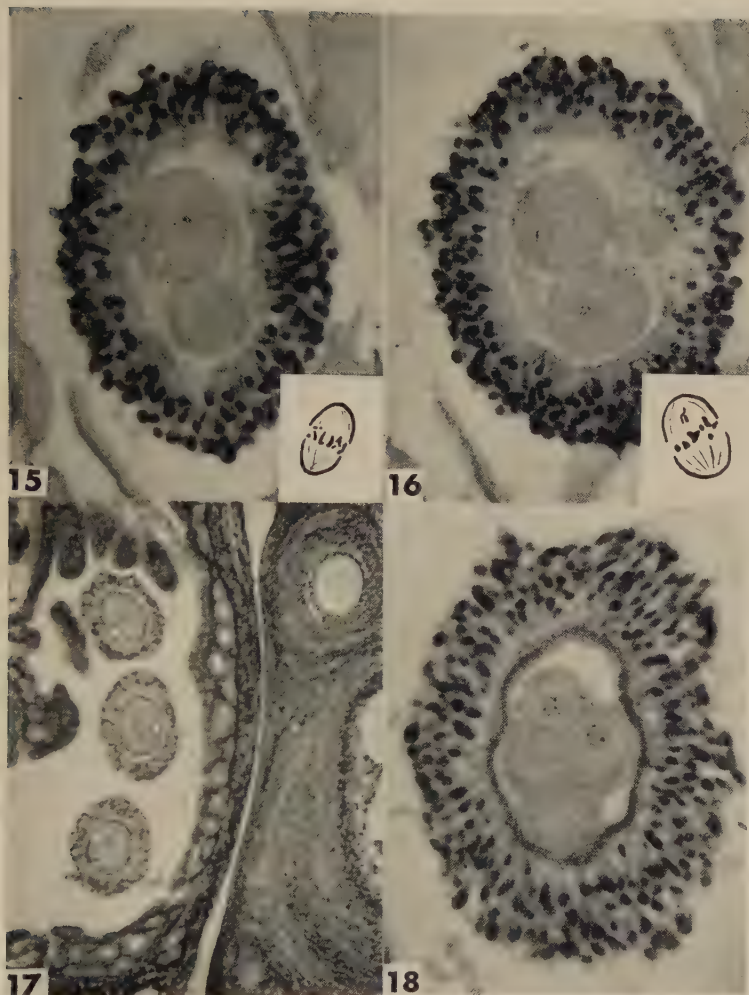
EXPLANATION OF FIGURES

Photomicrographs of sections of unfertilized ferret ova. All photographed with 4 mm objective, $\times 255$, except figure 17 which was photographed with 16 mm objective. $\times 59.5$.

15 and 16 Two sections of a 5-celled ovum, two cells with spindle.

17 One section of three ova in the tube.

18 One section of 6-celled ovum in tube. Normal nucleus in two cells.



THE SELECTIVE STOPPAGE OF BONE GROWTH IN TISSUE CULTURE ¹

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ONE FIGURE

1

INTRODUCTION

The culturing of bone tissue in vitro offers an excellent method for studying the effects of substances on bone growth. The method is not an easy one, however, and is complicated by the necessity of serially sectioning and staining the cultured tissue before one can decide whether bone growth has been enhanced or inhibited. With the hope of conserving considerable time the successful attempt was made to adapt the alizarin technique (Cameron, '30; Schour, Hoffman, Sarnat and Engel, '41; Ercoli and Lewis, '43) to the study of bone tissue in vitro. We were surprised to learn however that alizarin can act not only as a highly selective dye enabling one to determine easily the extent of ossification but, possibly even more important, that it can also act to stop all osteogenetic activity. This stoppage is selective. Other tissues in the preparation continue to grow normally.

TECHNIQUE

General procedure

In growing the bones the essential feature of Fell's ('28; '31) original technique was followed, i.e., place them on the surface of a clot. Metatarsal bones of 10 day old chick em-

¹This investigation was supported in part by a research grant (H-321) from the National Heart Institute, U. S. Public Health Service.

bryos were placed on clots held in the gutters of 32 cm³ vials laid on their sides (Paff, '48; Paff and Seifter, '50). Each tube contained about 2.0 cm³ of medium consisting of one part of blood plasma and one part of a mixture of embryonic extract and Tyrode solution. Four bones were placed in each tube and in one-half of the tubes alizarin red S had been added to the embryonic extract-Tyrode solution.

Certain precautions must be exercised in preparing the extract-Tyrode-alizarin solution. A 1% solution of alizarin water is first prepared. This saturated solution (not all of the alizarin will dissolve) is sterilized by passing through a filter, such as a Seitz. The filtrate serves as a stock solution and a few cubic centimeters will last for months.

The amount of stock alizarin solution used in making the mixture is very small and must be added carefully to prevent the formation of a heavy precipitate. One drop of stock alizarin solution is added to 59 drops of Tyrode solution while the latter is being vigorously shaken (a pipette which delivers small drops should be used). An easy way to assure success is to put the Tyrode solution in a 5 inch test tube and then carefully place one drop of alizarin high up on the wall of the tube. The tube is shaken vigorously while the dye is permitted to run down the tube wall into the Tyrode solution. Even so a fine flocculent precipitate may still form when an equal volume of embryonic extract is added to the Tyrode-alizarin solution. This need not worry the experimenter however if it is not permitted to settle to the bottom because when the extract-Tyrode-alizarin mixture is added to blood plasma the precipitate dissolves.

Design of the experiments

Two experiments were planned to explore the effect of alizarin red S on growing bones. In the first experiment 16 pairs of isolated metatarsal bones were cultured for 5 days without a change of medium. One member of each pair was cultured on medium containing alizarin whereas the other

was cultured on alizarin-free medium as a control. The total length of each bone collar was recorded for those which were placed on alizarin. At the end of 5 days 30 drops of extract-Tyrode-alizarin solution were added to each control tube as well as to those which already contained alizarin. Six hours later the lengths of all collars were recorded and the experiment was terminated.

In the second experiment 8 pairs of metatarsals were prepared as indicated in the experiment above but the next day the bones growing on the dye containing media were removed to a dye-free medium. At the end of 4 more days 30 drops of extract-Tyrode-alizarin solution were added to each tube for the purpose of staining all bone present, and the experiment was terminated.

OBSERVATIONS

Composition of a metatarsal bone

A metatarsal bone of the 10 day old chick embryo consists of three types of tissue namely, cartilage, bone and fibrous connective tissue. Cartilage is present in greatest amount. It forms the end of the bones and the great bulk of the shaft. The metatarsal bone at 10 days is little more than a cartilage miniature. A small amount of primitive bone tissue is present as a napkin-ring-like collar of tissue restricted to the middle third of the shaft. Bone trabeculae are almost always present. The range in trabeculation is indicated by the first and 6th pairs of metatarsals shown in figure 1 (1 C and 1 A; 6 C and 6 A). Finally the fibrous connective tissue is present as a membrane surrounding both the cartilage and bone. Adherent to the membrane is an additional amount of connective tissue which varies with the dissecting dexterity of the experimenter.

INCREASE IN LENGTH OF BONES

Table 1 presents the over-all lengths of the 16 pairs of metatarsal bones from 10 day old chick embryos used in the first experiment. All measurements are expressed in millimeters.

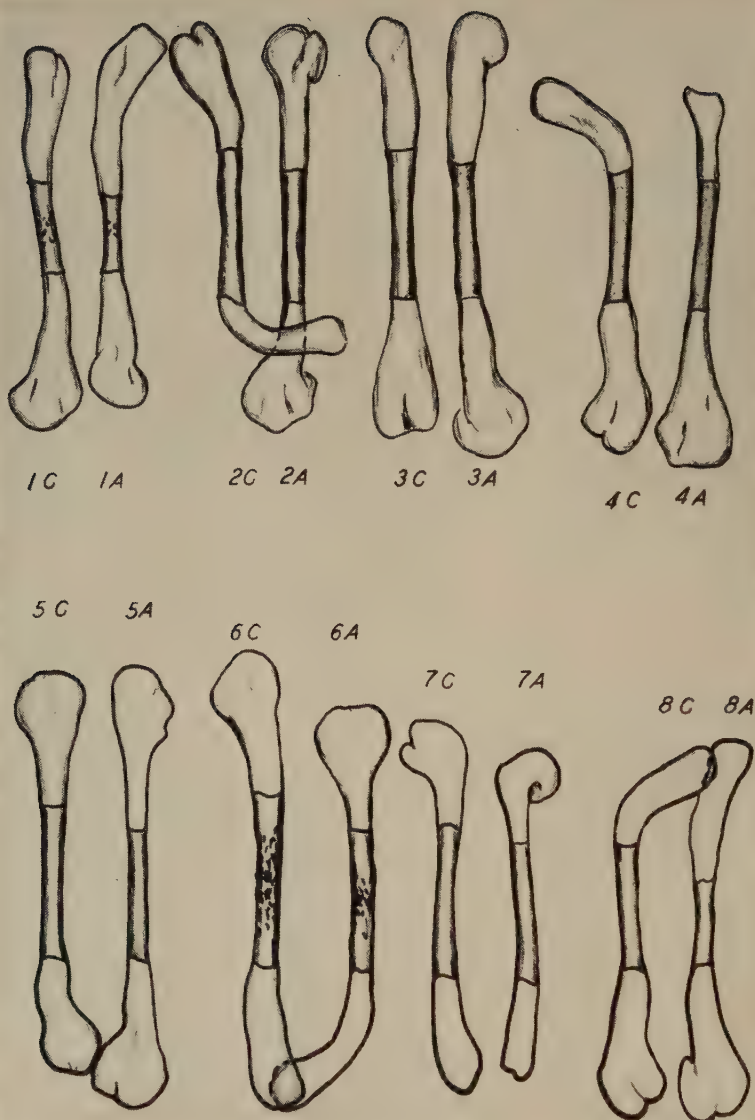
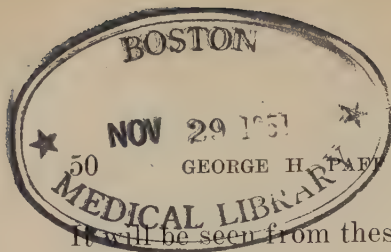


Fig. 1 Projection drawings of 8 pairs (1C and 1A; 2C and 2A; etc.) of metatarsal bones from 10 day old chick embryos. Bones 1C to 8C are controls grown for 5 days on alizarin free medium and then dyed with alizarin; bones 1A to 8A are "alizarin bones" grown for 5 days on medium containing the dye. The drawings indicate the overall size of each metatarsal and the extent of the bone collar. In only two pairs of metatarsals, namely the first (1C and 1A) and the 6th (6C and 6A) are the bone trabeculae included. These indicate the range of trabeculation observed. No attempt was made to outline the extent of fibrous connective tissue growth.

Note that in every pair the size of the bone collar is larger in the control than in the alizarin treated metatarsal. $\times 10$.

TABLE 1
Over-all lengths of bones
 (See text for explanation)

PAIR	BONES	LENGTH AT START	LENGTH AFTER 5 DAYS	INCREASE IN LENGTH
		<i>mm</i>	<i>mm</i>	<i>mm</i>
1	Control	5.75	6.50	0.75
	Alizarin	5.60	6.50	0.90
2	Control	5.25	6.85	1.60
	Alizarin	5.00	7.00	2.00
3	Control	5.00	6.65	1.65
	Alizarin	5.25	6.50	1.25
4	Control	5.20	7.00	1.80
	Alizarin	5.00	6.25	1.25
5	Control	4.65	6.10	1.45
	Alizarin	4.50	6.40	1.90
6	Control	4.70	6.20	1.50
	Alizarin	4.75	6.50	1.75
7	Control	4.25	6.25	2.00
	Alizarin	4.70	7.00	2.30
8	Control	5.20	7.00	1.80
	Alizarin	5.20	6.25	1.05
9	Control	4.50	6.00	1.50
	Alizarin	4.50	5.80	1.30
10	Control	4.95	6.20	1.25
	Alizarin	5.10	6.30	1.20
11	Control	5.50	6.50	1.00
	Alizarin	5.50	6.75	1.25
12	Control	4.75	5.75	1.00
	Alizarin	4.80	5.90	1.10
13	Control	5.35	6.60	1.25
	Alizarin	5.30	6.70	1.40
14	Control	5.40	6.80	1.40
	Alizarin	5.40	6.75	1.35
15	Control	4.75	5.90	1.15
	Alizarin	4.75	5.65	.90
16	Control	4.50	5.90	1.40
	Alizarin	4.35	5.75	1.40
Average	Control	4.98	6.39	1.41
	Alizarin	4.98	6.38	1.39



GEORGE H. PAPER AND FRANCIS C. EKSTEROWICZ

It will be seen from these data that the lengths of the alizarin treated bones are practically identical with those of the controls. The average values show this uniform length especially well. Thus the average increase in length of the metatarsals was 1.41 mm for the controls and 1.39 mm for those growing on medium containing alizarin.

THE BONE COLLAR

Staining and measurement

When isolated living bones are placed on clotted medium containing alizarin red S the selective action of the dye for the "calcifying or calcio-receptive zone" (Schour et al., '41) of the bones is not apparent for some time. A good result can usually be obtained in about two hours. If present the bone collar is then stained for as many days as the bone is cultured.

The earliest collar is represented by a diffuse red zone which is barely visible. In older collars, such as those seen in metatarsals from 10 day old embryos there is a diffusely dyed red zone. Bone trabeculae are usually visible within this deep red zone.

In table 2 the bone collar lengths of the 16 pairs of metatarsals are given. These are the same bones for which overall lengths were presented in table 1. In the last column of table 2 listing "increase in length" of bone collars the control values are qualified by a question mark. These values are based on the assumption that the invisible and unstained control collar at the start of the experiment was the same size as its alizarin dyed mate. To obtain the value of 0.55 (first figure given in the last column of table 2) we took the length of the control collar after it was stained at the end of 5 days (2.55) and subtracted the length of the alizarin bone collar at the start of the experiment (2.00).

In every case the length of the bone collar of the alizarin dyed bone was smaller than its control (fig. 1). Indeed the average increase in length of the dyed collar was so slight as

TABLE 2
Bone collar lengths
 (See text for explanation)

PAIR	BONES	LENGTH AT START	LENGTH AFTER 5 DAYS	INCREASE IN LENGTH
		<i>mm</i>	<i>mm</i>	<i>mm</i>
1	Control	Not dyed	2.55	0.55?
	Alizarin	2.00	2.20	0.20
2	Control	Not dyed	2.75	0.80?
	Alizarin	1.95	2.00	0.05
3	Control	Not dyed	2.75	1.00?
	Alizarin	1.75	1.85	0.10
4	Control	Not dyed	2.90	1.00?
	Alizarin	1.90	1.90	...
5	Control	Not dyed	2.00	0.50?
	Alizarin	1.50	1.45	— 0.05
6	Control	Not dyed	1.90	0.40?
	Alizarin	1.50	1.50	...
7	Control	Not dyed	2.20	0.80?
	Alizarin	1.40	1.45	0.05
8	Control	Not dyed	2.40	0.40?
	Alizarin	2.00	2.00	...
9	Control	Not dyed	1.50	0.25?
	Alizarin	1.25	1.25	..
10	Control	Not dyed	2.50	0.40?
	Alizarin	2.10	2.10	...
11	Control	Not dyed	2.30	0.05?
	Alizarin	2.25	2.20	— 0.05
12	Control	Not dyed	2.55	0.40?
	Alizarin	2.15	2.15	...
13	Control	Not dyed	2.50	0.35?
	Alizarin	2.15	2.15	...
14	Control	Not dyed	2.75	0.45?
	Alizarin	2.30	2.25	— 0.05
15	Control	Not dyed	2.50	0.35?
	Alizarin	2.15	2.15	...
16	Control	Not dyed	1.90	0.60?
	Alizarin	1.30	1.30	...
Average	Control	Not dyed	2.37	0.52?
	Alizarin	1.85	1.87	0.02

to justify the statement that no change had occurred. In contrast the bone collars of the control specimens demonstrated varying amounts of growth. This was evident not only by the increased length of the bone collar, but also by the appearance of new bone trabeculae.

TABLE 3
Bone collar lengths
(See text for explanation)

PAIR	BONES	LENGTH AT START	LENGTH AFTER 5 DAYS	INCREASE IN LENGTH
		mm	mm	mm
1	Control	Not dyed	3.25	1.10?
	Alizarin	2.15	2.15	...
2	Control	Not dyed	3.25	0.85?
	Alizarin	2.40	2.30	— 0.10
3	Control	Not dyed	3.15	0.90?
	Alizarin	2.25	2.25	...
4	Control	Not dyed	2.75	1.00?
	Alizarin	1.75	1.80	0.05
5	Control	Not dyed	3.10	1.10?
	Alizarin	2.00	2.05	0.05
6	Control	Not dyed	3.10	1.25?
	Alizarin	1.85	1.75	— 0.10
7	Control	Not dyed	2.40	1.30?
	Alizarin	1.10	1.10	...
8	Control	Not dyed	2.25	1.00?
	Alizarin	1.25	1.20	— 0.05
Average	Control	Not dyed	2.91	1.06?
	Alizarin	1.84	1.83	— 0.01

The results of the second experiment are shown in table 3. This experiment was done because it was felt that the failure of the bone collar to grow in the alizarin bones may have been due to their exposure to the dye for 5 days. Table 3 lists the lengths of bone collars from metatarsals in which the alizarin dyed bones were removed at the end of one day

from dye-containing medium to a dye-free medium. They were then cultured for an additional 4 days on dye-free medium. As shown in the table the control bone collars grew in length while the alizarin dyed bone collars remained static. Although the over-all lengths of the bones are not given the values revealed no significant difference between the control and dyed bones. The fibrous connective tissue growth was uniform in both the control and dyed specimens.

DISCUSSION

Of the three types of tissue found in the cultures only the bone tissue failed to grow when the medium contained alizarin. In preliminary experiments we obtained growth when the concentration of alizarin was considerably less than that used in the present investigation. Thus it appears that the reaction is directly proportional to the concentration of alizarin. If the concentration is too great growth is stopped in all the tissue elements, i.e., bone, cartilage, and fibrous connective tissue.

In marked contrast to the cessation of growth of bone tissue under the experimental conditions described, it was found that the cartilage and fibrous connective tissue remain quite unaffected by alizarin. In the case of cartilage this is well illustrated by the over-all length of bones listed in table 1. In reality these are values for hyalin cartilage since the great bulk of the bone is cartilage. No marked difference is to be found between the over-all lengths of control and dye-treated metatarsals. Measurements were not made of the fibrous connective tissue growth since it was so variable in its extent. In some members of a pair, the connective tissue of the alizarin preparation grew better than its control and vice versa. In no cases was there any indication of retardation of connective tissue growth.

The results listed in table 1 as well as the findings tabulated for bone collar lengths in tables 2 and 3, indicate that when applied to bones growing in tissue culture, alizarin red S can be used as a research tool in two entirely different ways.

The first of these is the conventional one of determining the extent of ossification by the simple procedure of staining the bones with alizarin at the end of an experiment. The second is to use the dye to block selectively the process of osteogenesis in one member of each pair of bones at the start of an experiment.

The mechanism whereby alizarin blocks the process of osteogenesis is unknown. It is not due to the fact that alizarin forms a calcium lake in the medium since removal of a metatarsal whose bone collar is stained, to a dye free medium does not result in the production of additional bone. It may be due to the formation of a non-utilizable calcium lake within the cells responsible for the formation of bone. This statement may have some justification since under phase microscopy one can find a goodly number of cells in the general area of osteogenetic activity which have coarse granules in their cytoplasm. Pollack ('28) was able to demonstrate the formation of purplish red granules in amebas injected with alizarin.

Whatever the reason for the cessation of bone formation the implications are most intriguing. Here is a substance which is specific in its combining action and which is non-toxic for other tissues at concentrations which completely inhibit the activity of bone forming cells. Could the same effect be obtained *in vivo*? Would it be possible to flood an animal with alizarin to the extent that all osteogenetic activity would cease? Could one arrest the growth of bone tumors by use of alizarin injection or ingestion? It is hoped that such problems may be answered by investigations which are now in progress.

SUMMARY AND CONCLUSIONS

Isolated metatarsal bones of 10 day chick embryos growing for a period of 5 days on medium containing alizarin red S have demonstrated the following:

1. Of the three types of tissue present, namely, cartilage, fibrous connective tissue and bone, only the bone tissue stains selectively.

2. The growth of cartilage and fibrous connective tissue is unaffected by the dye. In contrast all osteogenetic activity ceases. This is true whether the bones are grown continuously on medium containing the dye, or if the dye treated bones are later transferred to, and grown on, dye-free medium.

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THE DISTRIBUTION OF CHOLINE ESTERASE IN NERVE TISSUE OF THE DOG ¹

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TWELVE FIGURES

INTRODUCTION

The following observations are initial results obtained with the Gomori ('48) technic which permits the identification of sites of choline esterase activity in tissue sections. Their significance would be considerably enhanced if we could interpret these sites of enzyme activity as revealing true or specific cholinesterase which is considered to play an important role in nerve conductivity. Unfortunately there is evidence in the work of the original author (Gomori, '48), as has been noted by others (Koelle and Friedenwald, '49), that the specificity of the reaction may not be confined to true cholinesterase, but may be revealing sites of pseudo- or unspecified choline esterases. This lack of specificity does not in itself invalidate the usefulness of the technic in contributing to further data on the histochemistry of nerve tissue. In fact there is some evidence that unspecified choline esterases may be playing an important role in nerve conductivity (Koelle, Koelle and Friedenwald, '50).

METHODS

The technic as described by Gomori ('48) has been followed in essentially every detail. Very consistent results have been obtained on some 200 blocks of tissue collected from 10 dogs. Occasional erratic or incomplete tissue reactions were

¹ This work has been supported by a contract from the Office of Naval Research.

usually due to insufficient attention being paid to either the concentration or pH of the substrate, and repeated trials were sometimes necessary in order to obtain positive results in duplicate. Much of this difficulty was removed by reducing the concentration of the substrate to rather low levels. It is well founded (Mendel and Rudney, '43) that specific cholinesterase is easily inhibited by excess substrate (acetylcholine) and the inhibition is apparently due to choline (Roepke, '37). The substrates which we prepared for use in this technic were found to contain in solution quite measurable amounts of free acid and choline. Our percentage of positive reactions were immediately increased by using the substrate in concentrations ranging from 4×10^{-5} M to 12×10^{-5} M. Sections were prepared at 10μ and routinely incubated at 37°C . for from 16 to 24 hours. Control slides were prepared by the addition of 10^{-5} M of prostigmine to the substrate which served to inhibit completely the reaction in all nerve tissue.

Lauroyl-, myristoyl-, palmitoyl-, and stearoyl choline chloride were prepared essentially as directed by Gomori ('48). Maximum hydrolysis was obtained from the myristoyl and lauroyl salts with no discernible differences resulting in the intensity of the reaction (compare figs. 5 and 6). Nerve tissue of the dog failed to hydrolyze either palmitoyl- or stearoyl choline.

The authors gratefully acknowledge the assistance of Dr. George Gomori for a supply of myristoyl choline chloride and to Dr. A. M. Pardee and Mr. David Goodman for the preparations of other fatty acid salts used in this study. The technical assistance of Beryl Kallemeyn is gratefully acknowledged.

OBSERVATIONS

Spinal cord. Choline esterase activity is limited entirely to the gray columns with only the nuclei of some glial cells exhibiting a reaction in the white fasciculi. The anterior and

lateral columns consistently yield the maximum reaction (figs. 1, 2, 5, 6) while the central gray area and the dorsal horn are less reactive. The spongiosa is least reactive and occasionally negative. It seems clear that the site of enzyme activity is in the neuropil (figs. 5, 6) with the nerve cells exhibiting only a slight reaction and this usually confined to the peripheral cytoplasm (fig. 11). Nuclei of the nerve cells exhibit some reaction with the nucleolus highly reactive.

Evidence that the concentration of the enzyme, as indicated by the intensity of the reaction, is subject to variation depending upon the functional integrity of the nerve cells can be seen by examination of figure 3. The left sciatic nerve had been ligated in this dog 5 days prior to time of sacrifice. Choline esterase activity is abolished on the operative side and is somewhat reduced on the opposite side. In the latter site, where an enzyme reaction occurs it is localized in the same tissue elements as for normal cords. The effect of the nerve trauma appears to be one of reduction in enzyme concentration.

Brain stem. Maximal enzyme activity has been exhibited in all nuclear sites but considerably more intense in the motor nuclei. Thus, such nuclei as the hypoglossal (figs. 4, 10), vagal, and olivary nuclei are sharply delimited by virtue of a high choline esterase activity. Such interstitial, or receptive, nuclei as the gracile, cuneate, dorsal horn and spinal trigeminal are considerably less reactive but remain clearly identifiable from the surrounding white fasciculi. The response in the motor nuclei is essentially identical to that in the anterior column of the cord, namely an intense reaction in the neuropil with some tendency for localization around the nerve cells (fig. 10). Only slight activity is exhibited by the nerve cells save for the usual nuclear reaction (fig. 11). An exception is offered by the nerve cells of the olivary nuclei which show a marked enzyme activity equal in intensity to the reaction in the surrounding interstitial tissue (fig. 7).

Nerve fibers of such identifiable tracts as the pyramidal, medial longitudinal, spinal and medial lemnisci exhibit an

entirely negative reaction although nuclei of occasional glial cells are sharply reactive. Ependymal cells both of the central canal and chorioid plexus are negative. The area postrema does exhibit a very intense reaction.

Marked reactions were obtained for the thalamus and hypothalamus but the enzyme appeared not to be specifically associated with any discrete part. Various regions of the cerebral cortex proved non-responsive as was the case for the cerebellum. In the latter, however, a marked reaction was obtained for the dentate nucleus with the enzyme similarly distributed as in the brain stem nuclei and in the anterior horn.

Dorsal root ganglia. A rather scattered reaction is obtained for nerve cells, or the associated tissue, in sensory ganglia. Most striking is the intense reaction exhibited by the capsule surrounding the nerve cell bodies (fig. 9). That this reaction is not typical of all the cells is quite readily apparent (fig. 9) and in reviewing countless sections there is a distinct suggestion that the larger cell bodies are first, and more noticeably, reactive. The activity is expressed largely in the extracellular membranes (fig. 8). The prominent site of enzyme activity is immediately adjacent to the cell and can first be identified in the satellite cells and capsule. This location is clearly identifiable as a result of shrinkage of the cell thus leaving the reactive satellite cells clearly exposed at the periphery. Further, all degrees of reactivity can be identified varying from the minimal reaction in which only nuclei of satellite and capsular cells are "stained," thence an increase in reaction showing the capsule sharply outlined and, lastly, an intense reaction obscuring the tissue elements such as depicted in figure 8. The scattered nuclei of the supporting endoneurium always exhibit a marked reaction (fig. 8). The nerve cells exhibit a scattered cytoplasmic reaction and some nuclear activity particularly in the nucleoli (fig. 8).

Nerve fibers within the ganglia, as well as adjacent nerve roots, failed to exhibit any consistent activity. Rarely a slight precipitate occurs along the course of nerve fibers but it

has been impossible for us to ascertain if this reaction truly reflects enzyme activity within the fiber as distinguished from its possible location in sheath cells or endoneurium. Within the ganglia one can observe an occasional slight reaction within fibers as they originate from the cells. This is the only suggested evidence of an actual enzyme localization within the fiber.

Sympathetic ganglia. The site of enzyme activity varies in no essential manner from that described for sensory ganglia (compare figs. 8 and 12). A relatively slight reaction occurs within the cell but an intense reaction occurs extracellularly in the supporting tissue immediately surrounding the cell (fig. 12). In the latter position there is extreme variation in intensity of the reaction, it being limited in many instances only to nuclei of satellite and connective tissue cells. A distinguishable intracellular reaction is characteristically observed in the cytoplasm at the cell periphery but whether or not this reflects some diffusion from the usually intense reaction in the immediately adjacent supporting tissue is open to question. An occasional ganglionic cell does exhibit a diffuse cytoplasmic reaction but usually is not as intense as in the surrounding capsular area. Nuclei of the ganglionic cells are essentially negative save for an intense reaction on the part of the nucleoli.

DISCUSSION

A number of factors conspire to limit a proper assessment of the foregoing observations at this time. First, there remains considerable uncertainty as to the type of choline esterase responsible for exhibiting this reaction. That it may not be true or specific cholinesterase has been indicated by Koelle and Friedenwald ('49) and indeed Gomori ('48) reported an inability of a concentrated specific cholinesterase extract to hydrolyze the substrates used in the present technic. The latter author did get appreciable hydrolysis of these substrates from blood sera but in the presence of both specific and pseudo-cholinesterase. The former authors have em-

ployed a technic, differing from our own, which through the use of differential inhibitors appears to be capable of distinguishing between specific and pseudo- (non-specific) cholinesterases (Koelle and Friedenwald, '49). Descriptions of their observations are similar to our own, at least in many respects. Thus, these authors report "specific ChE only has been noted in and around the anterior and lateral horn cells of the spinal cord and in certain ganglion cells of the nodose and dorsal root ganglia." Such a localization would adequately describe our own observations (figs. 1, 5, 10, 11, for cord and motor nuclei, and figs. 8 and 9 for sensory ganglia). In sympathetic ganglia these authors also find specific cholinesterase associated with preganglionic fibers and we may possibly interpret the reaction with the present technic in a similar fashion (fig. 12). We have not obtained a sufficiently consistent reaction in protoplasmic tracts to permit a positive conclusion as have the authors of this other technic. Likewise, intense reactions within ganglionic cells have not been observed by us. The similarities in observations with the two technics appear to be sufficiently close to indicate the value of a simultaneous comparison of given tissue reactions but we have not been able to accomplish this at present.

Secondly, many deductions concerning the distribution of specific cholinesterase in nerve tissue have been made from quantitative assays of this enzyme (for review, Nachmansohn, '45). The localization of the reaction obtained with this histochemical procedure agrees in part with the quantitative conclusions but differences are encountered in many important respects. Thus, the intense histochemical reactions obtained for nuclear areas in cord and brain stem agree with the quantitatively high values for cholinesterase in these synaptic areas. However, a completely negative histochemical reaction for such tissues as peripheral nerve and roots, cortical areas, and to a lesser extent basal ganglia does not coincide with a considerable cholinesterase concentration in these sites. It must be recognized, however, that for interpretation of the histochemical preparations a negative reaction

does not necessarily prove the absence of the enzyme; it merely shows that under the conditions applied in the technic there is a failure to express a reaction. The localization of the reaction in sites previously recognized as yielding high cholinesterase values may be coincidence for the occurrence of an, as yet, unspecified choline esterase. It does seem clear that the enzyme is a choline esterase and not alone an unspecified esterase (Nachlas and Seligman, '49).

Quantitative determinations do not enable the precise definition of the tissue elements responsible for the enzyme activity. For example, in the gray columns the enzyme may be a product of either the nerve cells, synaptic terminals, glial components, or even free in the tissue spaces. Indeed, most if not all of these environments have been implicated with varying amounts of evidence (Nachmansohn, '45). It was to be hoped that the histochemical methods would precisely define the site of occurrence of the enzyme with customary allowances for technical artefacts. In this matter we have been somewhat disappointed through the circumstance that the available substrates which are reactive for dog tissue result in the formation of such a coarse precipitate that its localization in such finer elements as, for example, synaptic endings cannot be irrefutably defined (fig. 11). For sympathetic ganglia we might be justified in assuming that the prominent reaction at the periphery of the postganglionic cells (fig. 12) is a true expression of activity of preganglionic fibers and terminals were it not for two observations. First, some part of the reaction can be identified in the capsule, and second, this same localization occurs in dorsal root ganglia which are considered to be free of synaptic endings. Further, in a carefully executed experiment, Sawyer and Hollinshead ('45) were led to believe that the capsule on sympathetic cells was unaffected by their operative procedures and, by quantitative methods, deduced that it was not responsible for the production of either pseudo- or true cholinesterase. If the capsular reaction has been correctly interpreted by us then its occurrence in both sympathetic and sensory ganglia suggests that

we are dealing with an enzyme system which must be considered as playing some role in nerve metabolism in view of its intimate relation to the cell. The observation that nerve trauma results in an alteration in the intensity of the reaction (fig. 3) further suggests that both the presence and concentration of the enzyme are dependent upon normal nerve activity. At present it seems possible to conclude that a choline esterase capable of hydrolyzing myristoyl choline, and inhibited both by prostigmine and excess choline, is localized in many nuclear areas and points of synapse in both the central and peripheral nervous system. Further studies are in progress which are designed to identify more precisely both the tissue elements responsible for the enzyme reaction and the nature of the enzyme itself.

SUMMARY

The histological localization of a choline esterase which hydrolyzes both myristoyl and lauroyl choline chlorides has been determined for nerve tissue of dogs. In the central nervous system the enzyme reaction is almost entirely confined to the neuropil of the brain stem and cord. Nerve cells vary in the intensity of the cytoplasmic reaction but it is usually slight. Some enzymatic activity is exhibited by the cell nuclei and in particular the nucleoli. The white fasciculi of the central nervous system are essentially negative although the glial cells exhibit a positive reaction. Cortical areas of both cerebrum and cerebellum fail to yield a distinct reaction although the thalamus and cerebellar nuclei react to some degree. In the peripheral nervous system a prominent reaction is obtained for both spinal and sympathetic ganglia with the enzyme being localized at the periphery of the cells primarily in the capsular sheaths. Spinal nerves and roots are essentially negative but the supportive cells are positive.

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EXPLANATION OF PLATES

All figures are of dog tissue and show the sites of choline esterase activity in black. Myristoyl choline chloride was used as the hydrolyzable salt for all preparations excepting figures 1, 6, and 8 for which the lauroyl ester was used. The preparations were lightly counterstained in hematoxylin and eosin. Appropriate filters have been used so that only sites of enzyme activity are reproduced in the photographs as black. Both nerve cells and axons appear to be reactive in figure 6 but this is actually due to a heavy counterstain. In the preparation the white fasciculi are negative and the nerve cells quite comparable to those shown in figures 5 and 11.

PLATE 1

EXPLANATION OF FIGURES

- 1 Section of low cervical cord. $\times 6$.
- 2 High lumbar cord level, above the lumbar enlargement, from the same animal used for figure 3. $\times 6$.
- 3 The 7th lumbar level contributing to the lumbosacral plexus. Five days prior to sacrifice the sciatic nerve had been ligated on the left side. Note the complete loss of choline esterase activity on the affected side, and some reduction in activity on the contralateral side, as compared with the normal cord (fig. 1) or at unaffected levels above (fig. 2). $\times 6$.
- 4 Low medulla. The most intense activity is shown by such nuclei as the anterior olivary, hypoglossal, vagal, cuneate, gracile and, to a lesser extent, the dorsal column. $\times 4\frac{1}{2}$.
- 5 Anterior horn of cervical cord. An intense reaction occurs in the supporting tissue with some tendency to accumulate around the motor nuclei. The course of emerging motor fibers into the adjacent ground bundles is indicated by slight reactions along the course of these fibers. The constituent fibers of the ground bundles are negative. $\times 61$.
- 6 Area from a lateral gray column of the cord. Lauroyl choline chloride. $\times 61$.
- 7 Anterior olivary nucleus. Both nerve cells and interstitial substance exhibit an intense reaction (see also fig. 4). $\times 61$.

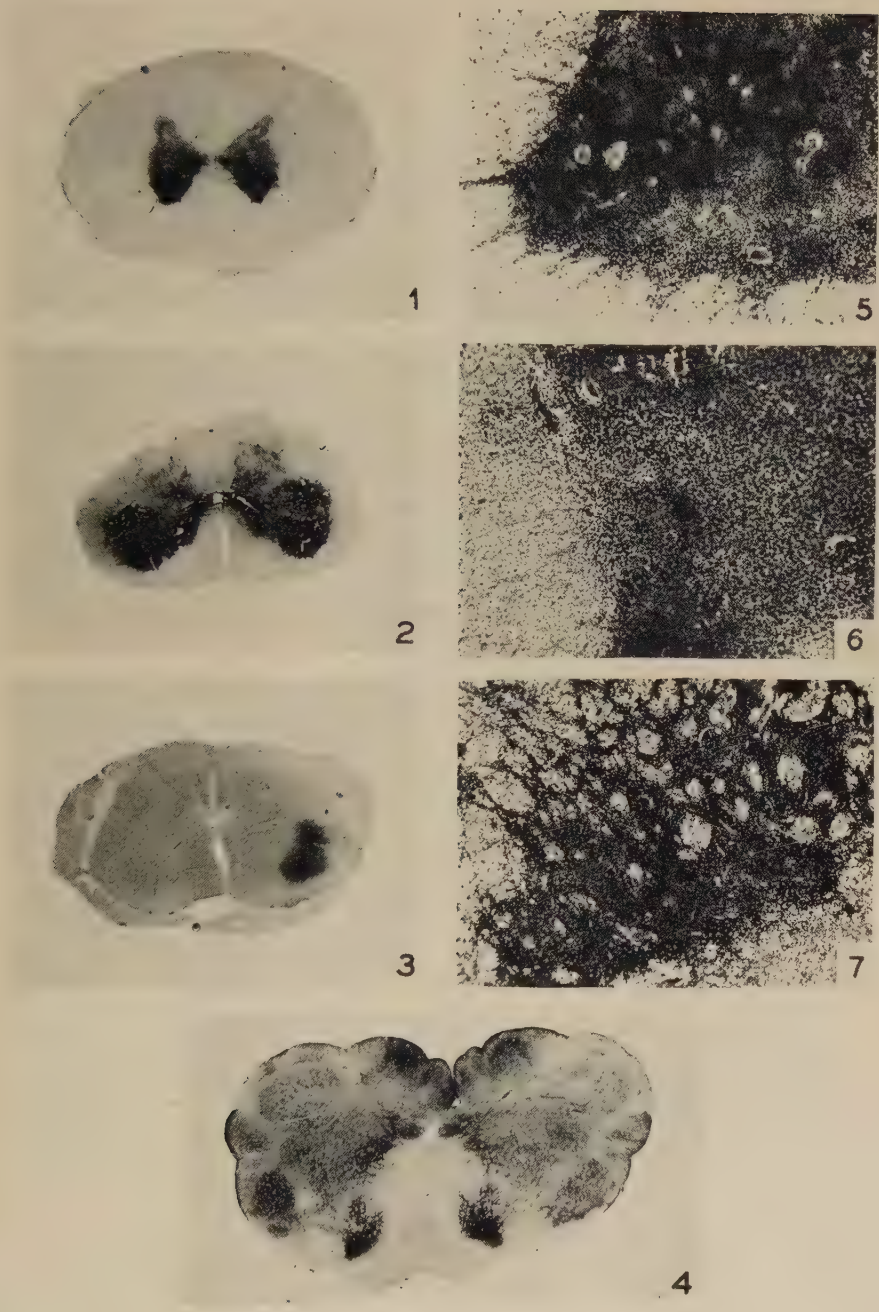


PLATE 2

EXPLANATION OF FIGURES

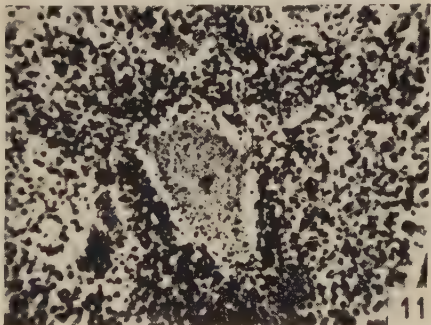
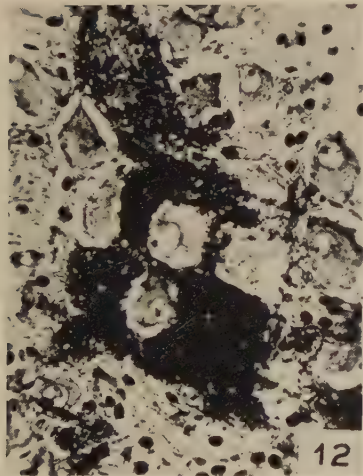
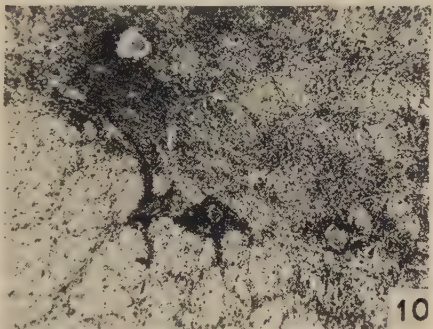
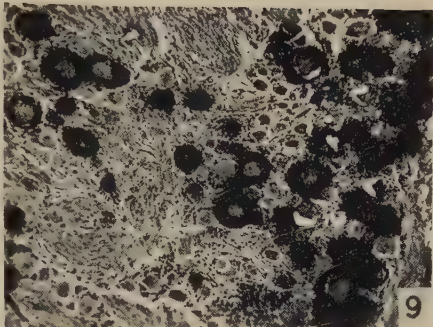
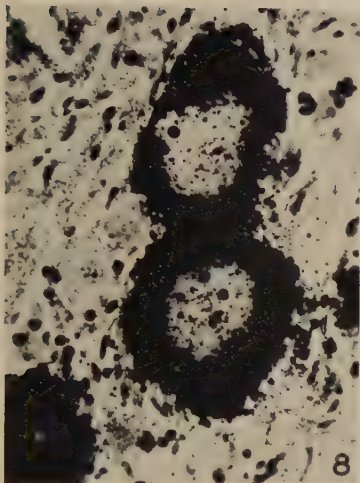
8 Dorsal root ganglion showing an intense reaction in the surrounding capsule. $\times 260$.

9 Dorsal root ganglion. Many of the nerve cells fail to show any marked reaction in the capsular membranes although both satellite and endoneurial tissue cell nuclei are very reactive. $\times 61$.

10 Area of the hypoglossal nucleus. Although the interstitial substance in the entire nuclear area exhibits a strong reaction there is a more noticeable concentration immediately around the motor cells. $\times 61$.

11 Anterior horn cell showing the nature of the reaction at the cell periphery. $\times 260$.

12 Superior cervical sympathetic ganglion. An intense reaction occurs in the capsule of some cells while in others only the capsular cell nuclei exhibit activity. $\times 260$.



THE DEVELOPMENT OF MOUSE OVA UNDER THE CAPSULE OF THE KIDNEY ¹

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THIRTEEN FIGURES

Since Brachet ('13) first observed growth in rabbit blastocysts explanted to plasma clots, numerous investigators have sought to determine to what extent mammalian ova and early embryos will undergo further development when removed from their normal association with the endometrium. In a previous paper we described the development of mouse ova in the anterior chamber of the eye and in the abdominal cavity. When transferred to these extrauterine sites, the ova implanted and gave rise to an abundant growth of placental trophoblast but there was relatively little differentiation of the inner-cell-mass (Fawcett, Wislocki and Waldo, '47). On the other hand, Nicholas ('42) reported that segmenting rat ova transplanted beneath the kidney capsule produced a rapid disorganized growth of various embryonic tissues but no visible differentiation of placenta or fetal membranes. The divergent results of these two investigations suggested that the kidney might provide an environment more favorable for the differentiation of the inner-cell-mass than that afforded by the anterior chamber of the eye. In the present study, therefore, we have extended our observations on the extrauterine implantation of mouse ova by exploring their potentialities for development under the kidney capsule.

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²John and Mary R. Markle Scholar in Medical Science.

MATERIALS AND METHODS

Segmenting mouse ova were successfully transplanted under the capsule of the kidney in 32 mice. No autotransplants were made. The host mice comprised 13 adult males, two immature males, two immature females, 7 pregnant females and 8 non-gravid adult females. The mice were of "Swiss" albino strain.

In order to obtain ova in the 4 to 8 cell stage, breeding females were killed on the second day after observation of the copulation plug. The oviducts were excised and transferred to a watch glass containing warm (37°C.) physiological salt solution. Under a dissecting microscope the coils of the oviduct were straightened out and the upper half discarded. The remaining segment was then stroked firmly with a flexible wire loop from the tubo-uterine junction upward so that the ova together with fragments of tubal mucosa were expressed into the surrounding fluid. To pick up and transfer the eggs, a glass pipette drawn from 3 mm tubing was used. This was connected by a short length of no. 10 rubber catheter to an Adams mechanical pipettor which was secured to the microscope or supported on a ringstand. This device produces pressure or suction in response to turning a knurled knob and provides somewhat finer control than is possible with mouth suction.

For transplanting ova to the kidney, the host mouse was anaesthetized with pentobarbital and the kidney was brought out through a small incision in the flank. A fine glass rod was inserted beneath the kidney capsule and swept from side to side in order to separate the capsule from the underlying cortex. A small subcapsular pocket was thus created into which the tip of the pipette was then introduced and the ova expelled.

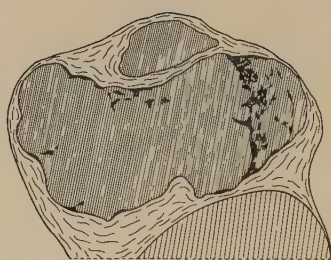
The mice were killed at various intervals ranging from 6 to 15 days after operation (1 to 17 days fertile age). The specimens were fixed in Zenker's fluid, embedded in paraffin, sectioned serially and stained with eosin and methylene blue or hematoxylin and phloxine.

OBSERVATIONS

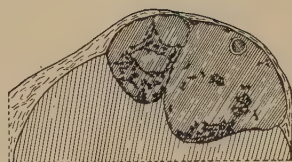
In being transferred to the kidney, eggs were sometimes damaged or were lost in the reflux of fluid through the incision in the renal capsule. To compensate for this loss and to improve the chances of a successful take, several ova were usually injected. It was not possible to see through the capsule well enough to identify and count the translucent ova against the mottled red background of the kidney cortex. The number of viable ova remaining under the renal capsule was therefore unknown, and hence neither the percentage of successful grafts within any one category nor the size of the resulting growths could be used as an accurate index for comparing results in hosts of different age or sex. However, successful grafts appeared to occur with about the same frequency and seemed to exhibit the same range of sizes whether the host was male or female, adult or sexually immature. Moreover, an established intrauterine pregnancy did not prevent the development of ova subsequently introduced under the capsule of the kidney.

The transplantation of ova beneath the kidney capsule was successful in approximately 60% of host mice of all categories. The resulting masses of fetal tissue varied in size from 1 or 2 mm up to a centimeter or more in diameter (figs. 2 and 3). A few grew to be even larger than the kidney. They were dark red in color, tense and fluctuant to touch. When studied in histological sections, the nature of the growth was found to be very similar to that previously described for ova developing in the anterior chamber of the eye (Fawcett, Wislocki and Waldo, '47). It usually consisted of a rather abundant growth of trophoblast, an abortive inner-cell-mass and a very marked extravasation of maternal blood. The relative proportions of these constituents varied considerably depending upon the age of the growth. Transplants of the same fertile age varied greatly in their development (fig. 1).

The extraembryonic ectoderm grew well for a week or two under the kidney capsule. Contrary to Nicholas' results



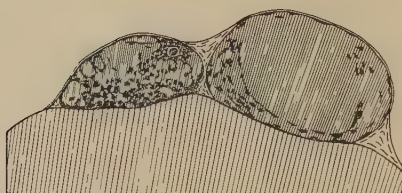
#11 adult ♂ 15 days



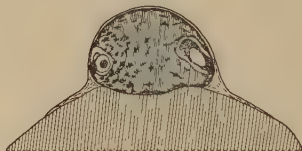
#25 adult ♀ 10 days



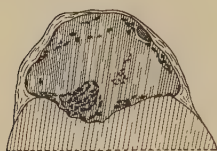
#3 adult ♂ 9 days



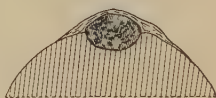
#5 adult ♂ 11 days



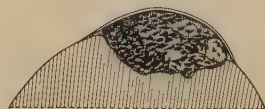
#12 adult ♂ 11 days



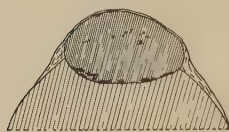
#29 immature ♀ 10 days



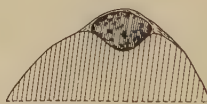
#22 adult ♀ 8 days



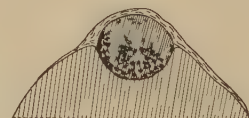
#16 immature ♂ 9 days



#14 adult ♀ 15 days



#28 immature ♀ 10 days



#24 pregnant ♀ 10 days

1

Fig. 1 Diagrams drawn by projection from typical histological sections of transplanted ova growing beneath the kidney capsule. The fetal trophoblast is indicated in solid black, extravasated blood by close cross-hatching and the kidney by coarser cross-hatching. It is apparent that grafts the same age vary greatly in size. The older growths, although they may be quite large, usually contain relatively little fetal tissue in comparison to the amount of extravasated blood.

with rat ova transplanted to this site, the inner-cell-mass did not flourish. Differentiation of recognizable tissues or organs of the embryo proper did not occur. In a number of instances, however, a yolk-sac of fairly normal configuration was developed (figs. 4, 5 and 9). It was set off from the surrounding trophoblast by a thin hyaline envelope corresponding to Reichert's membrane. Closely applied to the inner surface of this membrane were the flattened endodermal cells of the parietal layer of the yolk-sac. The visceral layer consisted of columnar cells with vacuoles and coarse eosinophilic granules in their apical cytoplasm similar to those found in the vitelline epithelium of the normal mouse placenta (Wislocki, Deane and Dempsey, '46). Capillary vessels and endothelial lined sinuses occasionally developed immediately subjacent to the visceral layer of the inverted yolk-sac and some of these contained primitive blood-forming cells (fig. 10).

Such well-developed yolk-sacs were not observed in the series of intraocular transplants previously reported. However, a frequent occurrence in those growths was a structure which consisted of a small aggregation of basophilic cells embedded in an amorphous eosinophilic matrix (figs. 6 and 7). Such objects were originally interpreted as an abnormal development of mesoderm in the inner-cell-mass. In the present series of kidney transplants similar formations were often found including some which were intermediate between these small masses of cells in matrix and easily recognizable yolk-sacs (fig. 8). It now appears probable that all such structures in both anterior chamber and kidney transplants actually represent efforts at yolk-sac formation. In the less successful ones a small group of endodermal cells appears to become embedded in an excessive elaboration of the amorphous material that normally forms Reichert's membrane (figs. 6 and 7). In the more successful, this material forms a delicate homogeneous membrane of the character of Reichert's membrane, around a yolk-sac provided with

typical vitelline epithelium and exhibiting areas of angiogenesis and hematopoiesis (fig. 10).

In reporting observations on rabbit blastocysts transplanted to the omentum, Waterman ('33) described a structure which is of interest when reviewed in the light of the present findings. This consisted of two highly convoluted layers of tissue, each one cell in thickness, enclosing a cavity filled with blood cells. The cells of the outer layer were columnar while those of the inner layer were flattened and had bulging nuclei. At one edge the two layers were continuous. Surrounding this body but separated from it by a space was a thin homogeneous membrane either collapsed or broken. Although different in their arrangement, the layers which he described correspond well with those we have interpreted as the parietal and visceral epithelia of the yolk-sac and Reichert's membrane as exemplified in figure 9 in the present study. Waterman, however, failed to identify these structures with the yolk-sac and found them so peculiar that he entertained some doubt that they were actually derived from the grafted blastocyst.

Transplanted ova 7 to 9 days fertile age growing under the kidney capsule consisted principally of rapidly proliferating trophoblast cells. The cytoplasmic processes of these cells communicated with one another to form a loose reticular syncytium enclosing an elaborate system of blood-filled spaces. This tissue resembled the trophoblastic labyrinth of an early intrauterine pregnancy. Mitotic figures were common among the smaller trophoblast cells at the center of such a growth but were very rarely found among the larger cells toward the periphery. The advancing margin consisted of giant trophoblast cells identical with those found at the junction of the fetal and maternal tissues in the normal mouse placenta. From the 10th day onward the rate of proliferation of the fetal tissue rapidly declined. The trophoblast cells seemed to lose the capacity for mitotic division and thereafter they underwent a progressive increase in size without further increment in their number. Thus, all of the trophoblast cells

were ultimately transformed into mononuclear giant cells. These cells comprised the major portion of the fetal tissue in transplants 10 to 12 days old (fig. 12).

Because of continued extravasation of maternal blood, the size of the masses on the kidney often increased for some time after the active growth of the fetal tissue itself had ceased. Thus, mice killed on the 14th or 15th day occasionally had a very large hemorrhagic mass on the kidney and considerable free blood in the peritoneal cavity. When sectioned, these large masses invariably proved to be little more than blood-filled cysts lined by a relatively small number of enormous trophoblastic giant cells (fig. 1, nos. 11 and 14). Clotting and organization of the extravasated blood appeared to be suppressed during the period of active proliferation of trophoblast, and invasion of the fetal tissues by maternal capillaries did not occur as long as any considerable number of viable trophoblastic giant-cells remained. After the 15th day, as the remaining giant cells regressed and finally disappeared, the accumulated blood gradually became organized by ingrowth of fibroblasts and capillaries from the underlying kidney cortex and from the thickened capsule. As replacement of the blood by connective tissue progressed, the grafts lost their hemorrhagic appearance and became lighter in color.

When multiple ova were successfully transplanted to the kidney, two frequently, and three occasionally, survived. In some instances discrete masses were formed a few millimeters apart. In others the surviving ova were near enough to one another that in their subsequent growth they coalesced into a single mass of tissue containing two or more centers of proliferation each with its own abortive yolk-sac (figs. 1 and 4). In a previous study (Fawcett, Wislocki and Waldo, '47), when multiple ova were transferred to the eye, they settled into the lowermost portion of the anterior chamber and began their development lying in very close proximity to one another. Under these conditions never more than one of the several ova continued to develop beyond the blastocyst

stage. It was suggested, therefore, that some product of the most precocious blastocyst suppressed the further growth of others nearby. No further evidence of such an effect of one developing blastocyst upon another could be adduced from the present experiments.

The anterior chamber of the eye provided very little room for developing blastocysts. Ova transplanted to the kidney, on the other hand, were easily able to grow outward, acquiring additional space by further separation of the capsule from the renal parenchyma. The kidney cortex was always somewhat indented by the growth. The fetal and maternal tissues, however, usually met in a curving unbroken line and the orientation of the adjacent renal tubules suggested that the deformity of the cortex was largely due to compression. On the other hand, in a few cases in the present study, the junctional zone was irregular and it was apparent that the expanding mass of fetal tissue was not only exerting pressure against the kidney cortex, but that the trophoblastic giant cells were also invading and destroying it. Giant trophoblast cells were occasionally found among the renal tubules some distance from the margin of the growth and isolated fragments of tubules were found in the midst of the fetal tissue, partially engulfed by giant cells (fig. 11). From time to time such cells were also observed to be partially or entirely within the lumen of small blood vessels in the kidney cortex, hence there could be no doubt that they were responsible for the escape of maternal blood into the intercellular spaces of the trophoblastic labyrinth.

In addition to their infiltrative and phagocytic properties, it has been repeatedly suggested that trophoblast cells elaborate cytolytic substances capable of causing tissue damage and extravasation of blood from vessels a short distance in advance of the margin of the fetal tissue. Transplantation of ova to the eye seemed to provide further evidence of such substances. The presence of trophoblast in the anterior chamber caused erythrocytes to collect in the posterior chamber and this blood apparently escaped from vessels not yet

in contact with the trophoblast. There was also marked edema and vascularization of the cornea which did not occur when other types of tissues were placed in the anterior chamber. It was anticipated that the kidney tissues would show a similar response to the growth of trophoblast under the capsule. On the contrary the kidney was relatively little affected. There was no interstitial hemorrhage within the kidney and the parenchyma adjacent to active growths of trophoblastic giant cells stained normally and showed no clear evidence of damage attributable to a cytolytic agent. Indeed, mitotic figures were not uncommon among the cells of renal tubules actually in contact with the margin of the fetal tissue (fig. 13).

DISCUSSION

In our investigations of extrauterine development, mouse ova have been transplanted to the anterior chamber of the eye, the abdominal cavity and the kidney. The kidney provided more space than the anterior chamber of the eye and the ova grew to a larger size and achieved somewhat more normal differentiation of the yolk-sac. Although the physiological conditions encountered by the ova in the several extrauterine sites studied were quite diverse, there was relatively little difference in the amount of growth or in the degree of differentiation attained. Hence, transplanted mouse ova in the early stages of their development appear to be relatively independent of local conditions and are capable of producing a growth of trophoblast, trophoblastic giant cells and an abortive yolk-sac in almost any environment which provides the minimum requirements for cell multiplication.

The fact that an established intrauterine pregnancy did not prevent the development of ova subsequently introduced under the capsule of the kidney is of particular interest in relation to the controversial subject of superfetation. It is well known that ovulation can be induced in pregnant animals (Wislocki and Snyder, '31), and estrus and copulation during pregnancy have been reported (Long and Evans, '22;

Nelson, '29). Nevertheless, the occurrence of true superfetation is still not generally accepted. It would appear from the results reported here that if ovulation and fertilization can be made to occur in pregnant animals then there is no hormonal effect of pregnancy which would act directly upon the ova to suppress their further development.

Ova transplanted to the kidney, like those developing in the anterior chamber of the eye, had a very limited life span. Active proliferation of the trophoblast continued for only about 12 days. Thereafter, growth ceased and the grafts gradually regressed. This corresponds well with the duration of placental growth in utero. In the course of a normal gestation the embryonic portion of the placenta is still growing on the 12th day but by the 15th day mitoses have ceased. The placenta has reached its greatest size by the 16th day (Bridgeman, '48).

In all of the extrauterine sites so far studied, transplanted mouse ova have caused extravasation of blood. This hemorrhage is attributed to the activity of the trophoblastic giant cells which opened maternal blood vessels and sometimes infiltrated deep into the tissues of the host. These giant cells are thought to play an important role in the normal implantation and growth of the blastocyst, by eroding and destroying the decidua. This is highly credible in view of their aggressive behavior toward extrauterine maternal tissues.

When grafts of tumors or embryonic tissues are introduced beneath the kidney capsule they are promptly vascularized by ingrowth of capillary vessels from the renal cortex. It is interesting that such was not the case when segmenting ova were transplanted to this site. Active proliferation of trophoblast appears to suppress the growth of fibroblasts and capillary endothelium which normally lead to vascularization and encapsulation of tissue grafts. Only after the involution of the trophoblast is well advanced do these processes gain ascendancy and gradually replace the growth with well vascularized maternal connective tissue.

Greene ('43) transplanted 6 to 8 day rabbit embryos to the anterior chamber of the eye and obtained grafts containing a disorganized growth of tissues representing all three germ layers. The tissues were generally well differentiated by the 10th day after transfer and in some instances the maturation in the anterior chamber appeared to be more rapid than in utero. Similar observations have been made on rat ova and egg cylinders transferred to the kidney and to gut segments denuded of their mucosa (Nicholas, '42, '50). In both of these sites growth and differentiation was said to be far in advance of what would be expected from the same length of incubation in the uterus.

In our experience with mouse eggs, the size of the growth on the kidney did sometimes exceed that of a uterine swelling of corresponding age. However, owing to the inclusion of rather large amounts of maternal blood, the size of the mass was a poor index of its content of fetal tissue. Furthermore, some of these masses represented the growth of more than one ovum. Studied in histological sections, the amount of fetal tissue developing from any one egg did not appear to be greater than normal. When a well-formed yolk-sac was found it usually corresponded in its development with that of a normal gestation of similar age. Hence, there was no evidence from the present study that the kidney offered a superior environment leading to accelerated growth or precocious differentiation.

Although similar experiments were done in very closely related species, our observations are at variance with those of Nicholas on several points. It is impossible to state at present whether the divergent results of these two investigations are due to species differences, to differences in the stage of development of the ova at the time of transplantation or to variations in technique. In Nicholas' experiments with rat ova there was an extensive and precocious development of embryonic tissues without differentiation of placenta or fetal membranes. The growths were well vascularized, showed no areas of diffuse hemorrhage and never actively

invaded the substance of the kidney. On the other hand, in the present study of mouse ova transplanted to the kidney, no tissues or organs of the embryo developed. Instead, differentiation of a yolk-sac and trophoblastic labyrinth took place at the normal rate. Associated with the formation of these placental elements there was extensive extravasation of maternal blood. The trophoblastic giant cells formed at the periphery of the growth eroded and infiltrated the kidney cortex.

Transplanted ova apparently can develop either into a disorganized mass of embryonic tissues and organs or into a growth consisting entirely of placental structures. Simultaneous differentiation of embryonic tissues and placental elements has not yet been observed in ova developing in extrauterine sites. A consideration of the results of the present investigation suggests an explanation for this. If the trophoblast flourishes, ingrowth of capillaries is suppressed. There is instead an extravasation of maternal blood which provides a nutrient medium which will support further growth of trophoblast and differentiation of a yolk-sac but is inadequate for the development of the inner-cell-mass. It has recently been shown that if the ectoplacental cone is removed from mouse egg cylinders before they are transplanted, trophoblastic giant cells are not formed, there is no hemorrhage and differentiation of the inner-cell-mass can occur (Grobstein, '49). Under the conditions of Nicholas' experiments the differentiation of trophoblast may have been suppressed. This would permit the ingrowth of capillaries from the highly vascular stroma of the kidney or the gut and allow a disorganized growth of various derivatives of the inner-cell-mass to proceed. For an orderly and integrated development of both embryonic and extraembryonic tissues the highly specialized environment of the uterus may be necessary.

In considering the two different types of growth resulting from the transplantation of ova it is interesting to recall that there are two corresponding types of ovarian tumors, thought to arise from included blastomeres or from partheno-

genetic development of ova. These are the solid teratoma, consisting of disorganized embryonic tissues and the chorion-epithelioma which consists of a tumorous growth of placental elements. There are scarcely any reports in the medical literature of tumors which contained both embryonic tissues and placental structures (MacCallum, '32).

SUMMARY

Segmenting mouse ova were transplanted beneath the kidney capsule in adult and immature mice of both sexes and in pregnant females.

The age, sex or physiological condition of the host had no apparent effect upon the success of the graft.

Growth of the ova produced hemorrhagic masses under the capsule which consisted principally of trophoblast, giant cells and extravasated maternal blood. In several instances yolk-sacs also developed. These were enclosed in a typical Reichert's membrane and consisted of two layers of epithelium which closely resembled those of the visceral and parietal layers of the normal yolk-sac.

Beginning angiogenesis and hematopoiesis were occasionally observed but differentiation of other tissues or organs of the embryo did not occur.

Ova transplanted to the kidney had a very limited life span. Proliferation of the trophoblast continued for only 12 to 14 days. This corresponds in duration with the growth period of the fetal placenta in a normal intrauterine gestation.

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PLATE 1

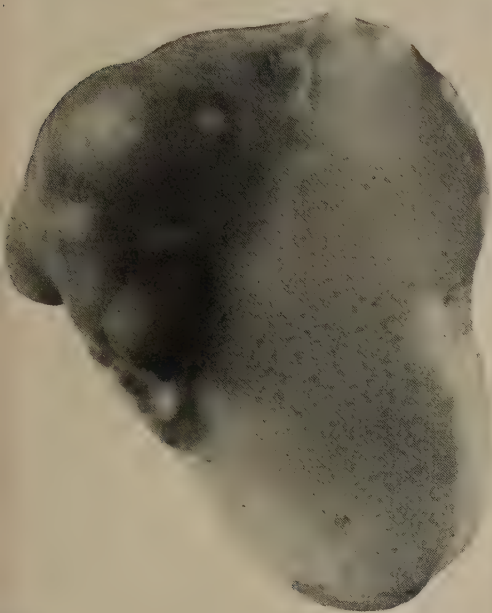
EXPLANATION OF FIGURES

2 A photograph of a mouse kidney 10 days after segmenting mouse ova were introduced beneath the capsule. Growth of these ova has produced a nodular hemorrhagic mass of fetal tissue and extravasated maternal blood. $\times 9$.

3 A smaller growth under the kidney capsule 8 days after transplantation of 8-cell eggs. $\times 9$.

4 A histological section through a mass which appears to have resulted from the development of two viable ova situated near one another under the kidney capsule. In their growth (11 days) the two have coalesced into a single mass in which there are two distinct yolk sacs. Eosin and methylene blue. $\times 45$.

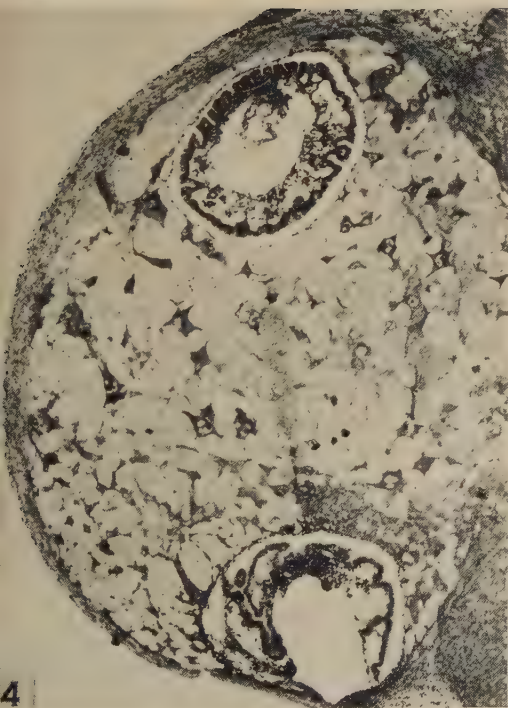
5 A typical inverted yolk sac separated by Reichert's membrane from a rather dense growth of large trophoblast cells (9 days fertile age). Eosin and methylene blue. $\times 90$.



2



3



4



5

PLATE 2

EXPLANATION OF FIGURES

6 and 7 Two structures which developed from ova implanted in the anterior chamber of the eye. These consist of basophilic cells imbedded in an amorphous eosinophilic matrix. One contains a layer of epithelium. These structures which were also found in transplants to the kidney are now interpreted as abortive yolk-sacs in which the endodermal cells have become enclosed in an excessive elaboration of the amorphous material which normally forms Reichert's membrane. Haematoxylin and eosin. $\times 200$.

8 A somewhat more successful attempt at yolk sac formation in a kidney transplant. In the upper half of the figure an incomplete Reichert's membrane of normal thickness is seen. Cells of the parietal layer of the yolk-sac adhere to the membrane and are separated by a narrow cleft from a disorderly growth of the visceral layer of vitelline epithelium. Eosin and methylene blue. $\times 330$.

9 Section through a growth 9 days old showing differentiation of Reichert's membrane and a rather normal appearing yolk-sac with typical visceral and parietal layers. The cavity enclosed by the visceral layer of endoderm may represent the amniotic cavity. Haematoxylin and phloxine. $\times 250$.

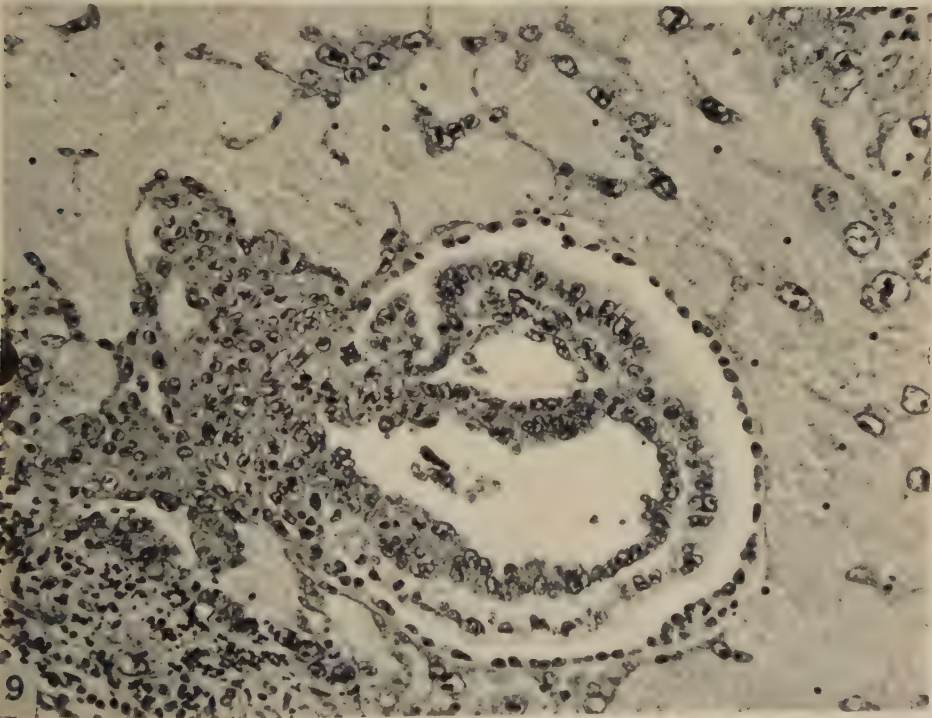
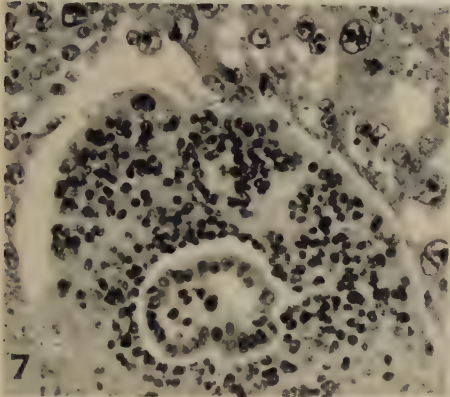
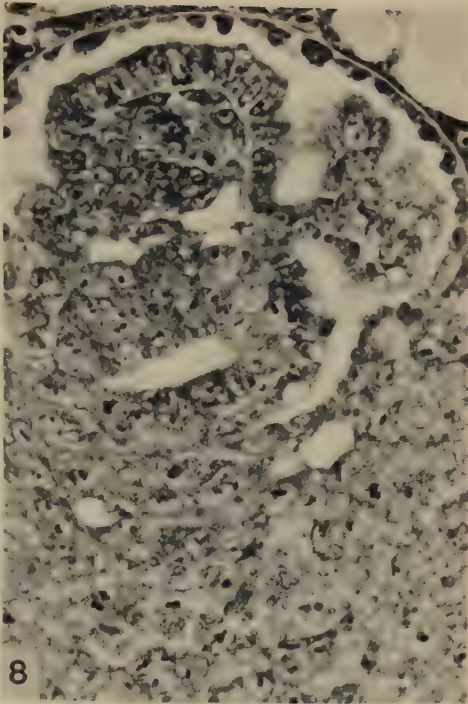
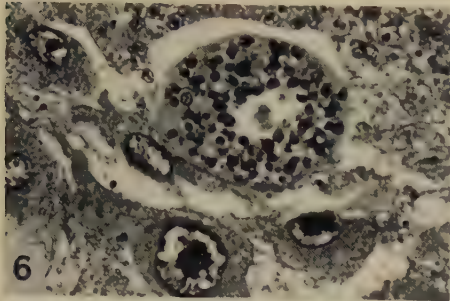


PLATE 3

EXPLANATION OF FIGURES

10 Section of the yolk-sac of a 9-day kidney transplant. The epithelium consists of cells with vacuoles and eosinophilic granules in their apical cytoplasm which closely resemble those in the visceral layer of the vitelline epithelium in a normal intrauterine gestation. Fetal capillaries are also developing and some contain primitive blood forming cells. Eosin and methylene blue. $\times 330$.

11 Drawing of the margin of the fetal and maternal tissues in a graft to the kidney showing invasive trophoblastic giant cells making their way between the tubules. At the left of the figure an isolated fragment of a tubule appears to be within the richly vacuolated cytoplasm of a giant cell. Camera lucida at $\times 500$.

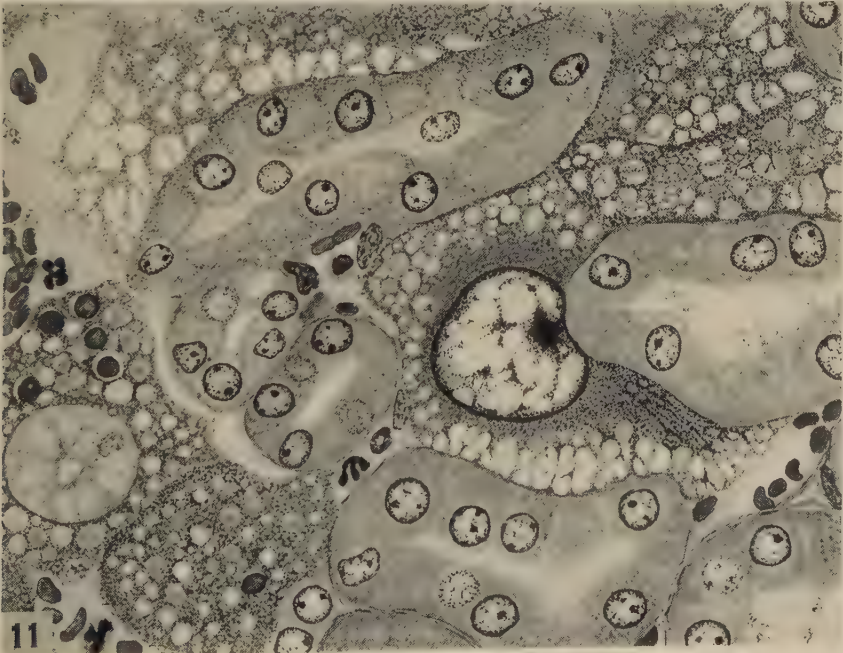
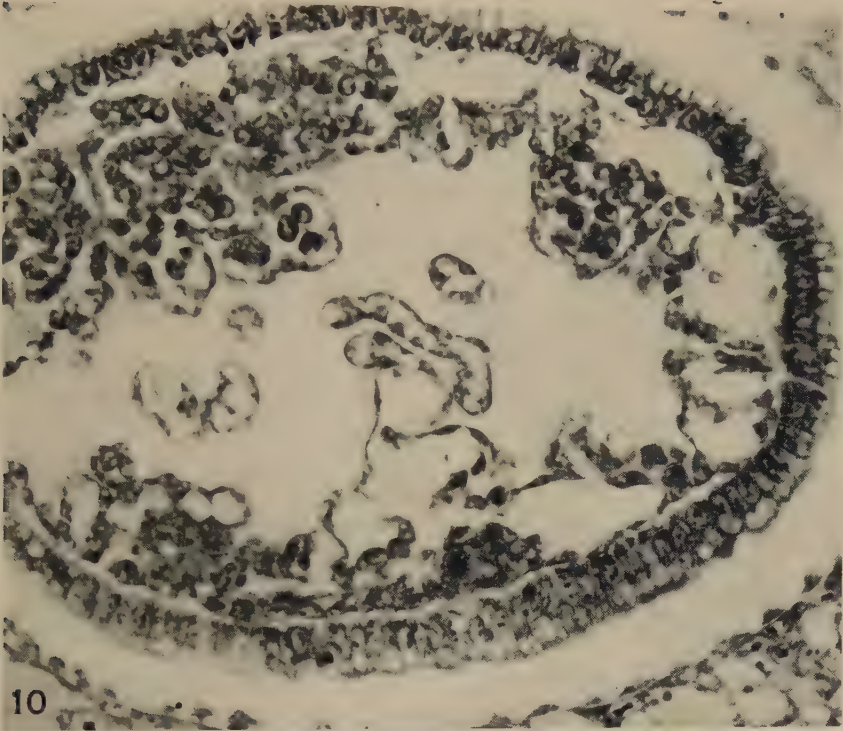
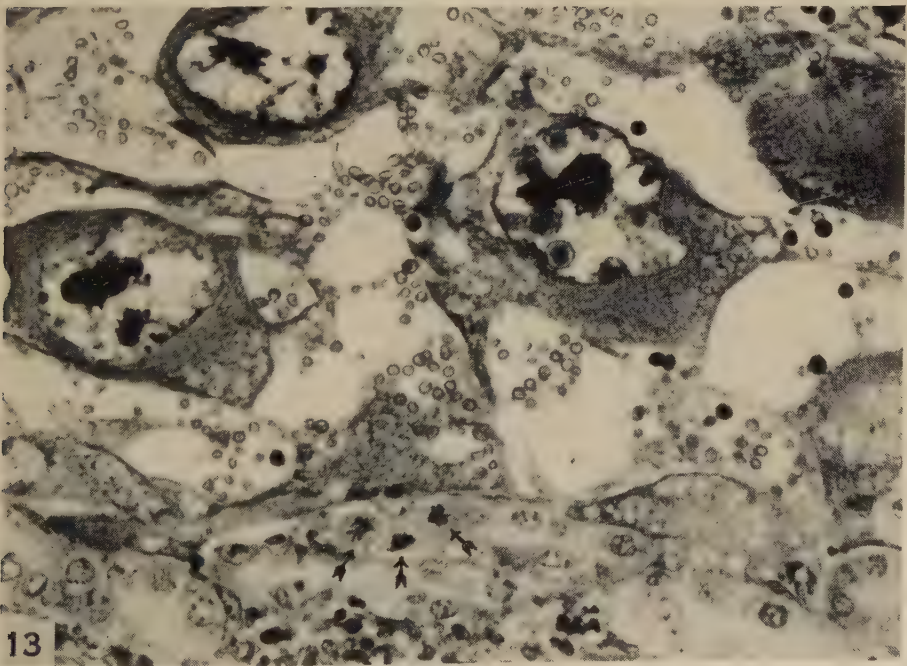
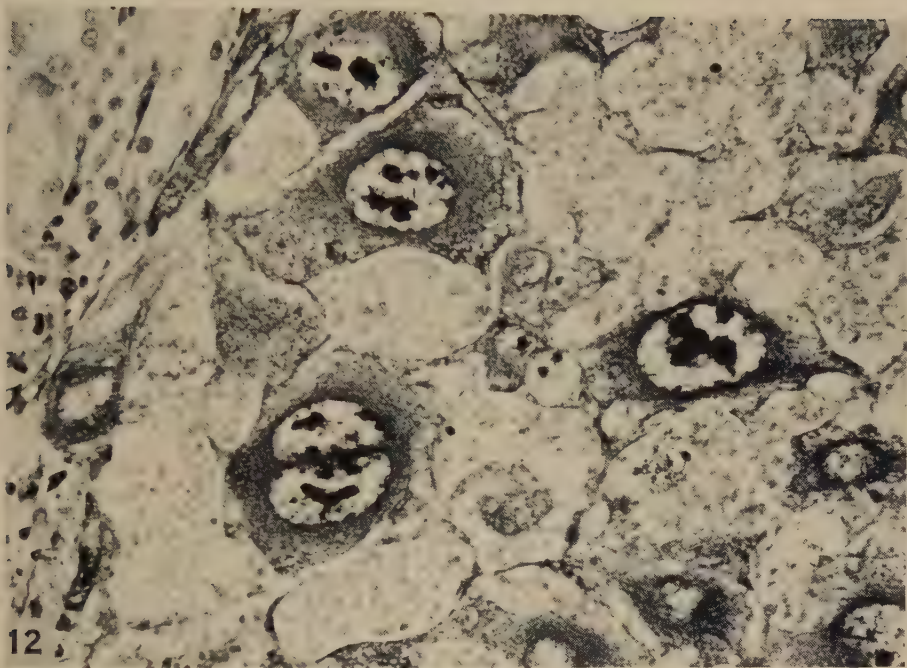


PLATE 4

EXPLANATION OF FIGURES

12 Trophoblastic giant cells with their cytoplasmic processes communicating with one another to form a loose reticular syncytium enclosing lacunae filled with maternal blood. Eosin and methylene blue. $\times 330$.

13 Photomicrograph of the junction of the fetal trophoblast with the kidney cortex. The tubules immediately adjacent to the giant cells stain normally and show no evidence of cytolysis. Three mitotic figures among the cells of a renal tubule are indicated by arrows in the lower part of the figure. Eosin and methylene blue. $\times 480$.



THE GROWTH OF HUMAN MYOMETRIUM AND ENDOMETRIUM

STUDIES OF CYTOLOGICAL ASPECTS

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NINE FIGURES

Recent cytogenetic research points to an intimate relation between nuclear volume and the number of chromosomes or genome,² that is, according to whether they are haploid, diploid or polyploid or whether the chromosomes are polytenic or simple. Recent karyometric statistical research shows that the interphasic growth of the nucleus occurs in steps, and that the final nuclear volume of each step is a multiple of the volume of the diploid genome, which therefore may be considered as the unit or basic value in the process of duplication. The karyometric statistical method thus offers one of the few possibilities for quantitative inquiry into the phenomena of reproduction of the genes (Schreiber, '47a).

In a preceding report (Salvatore and Schreiber, '47a) it is stated that variations of nuclear size in uterine tissues are typically rhythmical, i.e., they occur as multiples of a given basic value which indicates that such variations are effected through the reproduction of the nuclear genome. In the uterine muscle also, notwithstanding the rare incidence of

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² By the term *genome*, the geneticists understand: "Any groups of chromosomes composing a nucleus, whatever their number or kind, is a chromosome complement. The simplest typical complement is made up of several members differing variously in form and function but acting together as a complete and harmonious system; such a complement is a genome or set" (L. S. Sharp, '43, *Fundamentals of Cytology*, 3d. Ed., New York, pp. 121 and 353).

mitoses, there exist interphasic growth cycles of the nuclei, identical to those observed in endometrial epithelium and related to phases of the estrous cycle and pregnancy. In castrated female rats, also, estrone induces in the nuclei an interphasic growth, with doubling of their original volumes (basic volume).

In other reports, Salvatore ('48a, b) has emphasized the essentially chromosomal nature of nuclear hypertrophy, for karyometric statistical studies show that the increase of nuclear volume is due to a reproduction of the nuclear genome. This conclusion is also established by the incidence, in the myometrium of puerperal uteri of castrated rats (Salvatore, '49), of many mitoses, and of typical prophases in muscle cells with nuclei of large volume which indicate that these nuclei had grown in size during pregnancy, not through simple imbibition but because of phenomena of a genic nature.

TABLE 1

Human myometrium

Frequencies of nuclear volumes of muscle cells and their respective modal values, in both phases of the menstrual cycle and in two stages of gestation. Except where stated as μ^3 , modal values and volumes are as measured on tracing paper from camera lucida drawings, before conversion into microns.

N° OF PROT.	PHASES	MODAL VALUE					NUCLEAR VOLUME				
		I	II	III	IV	V	400	500	600	700	800
55	Prolif.	503	788	1100			13	27	15	16	24
100	Secret.	524	712	1110			13	98	37	65	62
57	4th month of pregnan.	540	712	1100	1534		1	16	9	25	51
75	9th month of pregnan.			1004	1412	1891				3	10
Average of modal value		522	767	1078	1473	1891					
Average in cubic micra		77.7	114.2	160.6	219.4	281.7					

A survey of the literature dealing with uterine cytology contains few references to the above mentioned subjects. The papers of Stieve ('29, '32), Gander ('30), Froböse ('34, '35) and Fabris ('35), describe only an increase in the length and width of nuclei of muscle cells during pregnancy, with no data as to the determination of nuclear volumes and their quantitative changes.

For the endometrium,³ O'Leary ('31) studied statistically the relation between cellular and nuclear volumes of human endometrial cells. He did not relate this to cytogenetics, however, and did not indicate the quantitative changes in the nuclei. During the first phase of the menstrual cycle, the endometrium constitutes a splendid material for study of the nuclear interphases induced by specific known factors; namely, the ovarian hormones.

³ While this paper was under consideration by the Editorial Board of The Anatomical Record, we had knowledge of a similar work by E. Hintzsche (Zyklische Änderungen der Kerngröße in Oberflächenepithel und Drüsen des menschlichen Uterus. Gynaecologia, 1949, 128: 270-285) dealing on the subject.

TABLE 1 (continued)

Human myometrium

NUCLEAR VOLUME														N° OF NUCLEI
1000	1100	1200	1300	1400	1500	1600	1700	1800	1900	2000	2100	2200	2300	
3	5	4	2	2										121
4	11	8	2	2	..	..	1							273
17	28	23	16	4	13	13	3	2	1					250
37	30	27	23	43	38	17	14	7	8	5	3	..	1	289

Presented in this report are the results of our karyometric statistical observations on muscular and endometrial tissues of the human uterus which will allow us to ascertain the existence or not of rhythmical nuclear growth.

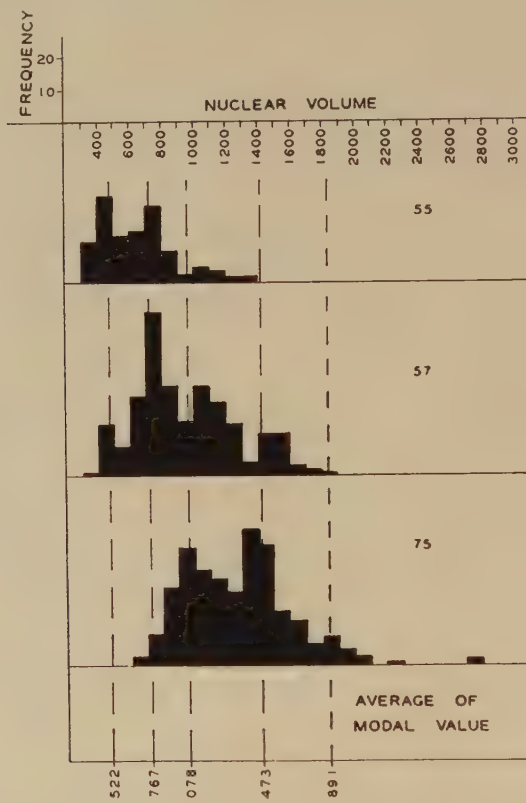


Fig. 1 Histograms showing the frequency of nuclear volumes of uterine muscle cells in the proliferative stage (record 55), in the 4th month of pregnancy (record 57), and in the 9th month of pregnancy (record 75).

MATERIAL AND TECHNIQUE

1. Myometrium. Bits of human myometrium were excised surgically during laparatomies. The specimens representing the two phases of the menstrual cycle (specimens 55 and 100) were obtained from women whose menstrual cycles were nor-

mal but who were operated on for uterine retroversion. The myometrial specimens of pregnancy were obtained at Caesarian sections.

2. Endometrium. Endometrial fragments were obtained by suction or curettage from healthy women exhibiting normal menstrual cycles. The specimens represent the following days of the menstrual cycle: days 5 (24 hours after the end of menstruation), 6, 10 (2 cases), 13, 14, 18, 19, 24, and 27. Thus the material consists of 4 samples each from the proliferative and the secretory phases, and two from the transitional phase.

TABLE 2

Myometrium

Average values of the length and width of muscle fiber nuclei, and their average volumes

N° OF PROT.	PHASES	MICRA LENGTH × MICRA WIDTH			N° OF THE NUCLEI	AV. VOLUME IN μ^3
		(average)		(average)		
55	Prolif.	17.5	×	3.0	25	82.5
100	Secret.	21.6	×	2.6	102	76.6
57	4th month of pregnan.	16.3	×	4.1	58	143.1
75	9th month of pregnan.	19.2	×	4.2	71	177.0

The histology of the sections agrees exactly with the predicted phase.

The material was fixed in Duboscq-Brasil or alcoholic Bouin fixative, imbedded in paraffin, cut at 10μ and stained with hematoxylin and eosin. The nuclei (200–600 in each sample) were drawn with the camera lucida at a magnification of 1890 diameters and their outlines measured on the tracing by the use of transparent millimeter paper. The nuclei drawn were chosen from regions in which the muscle fibers or the endometrial cells lay approximately parallel to the plane of section, to avoid inaccuracies due to foreshortening.

The statistical method employed in analyzing the measurements followed the principle used in a preceding paper (Salvatore, '48), and can be summarized as follows: The nuclear volumes were determined by the formula $V = \frac{a^2b}{1.91}$ (a being the minor diameter and b the major diameter of an ellipsoid) and the nuclei were grouped in frequency classes with intervals of 100 μ^3 . For convenience, the measurements used were those of the camera lucida tracing rather than the actual nuclear sizes in micra. To calculate the modal values, which represent the real maximal values of the nuclear volume frequencies, we used the following formula (Arkin and Colton ['42], page 23):

$$M = L + \frac{F}{F + f} \times i$$

where L is the inferior limit of the modal class, F is the frequency of the class superior and f that of the class inferior to the modal class, and i is the class interval. The modal values were converted into cubic micra.

RESULTS

1. Myometrium. Analysis of table 1 and the histogram of figure 1, shows that in both the proliferative and the secretory phases the nuclear volumes tend to fall in three groups of maximal frequency, with their modal values in the approximate ratios of 1:1.5:2. The nuclei representing mode I average 77.7 μ^3 in volume, which seems to be the basic value. The nuclei of mode II average 114.2 μ^3 , and those of mode III 160 μ^3 , the latter being a little more than double the first modal value. The nuclear volumes from the 4th month of pregnancy show the first three modes and a 4th mode whose nuclei average 219.4 μ^3 , three times that of the basic value. In the specimen from the end of pregnancy, the smaller nuclei of modes I and II are virtually absent, most of the nuclei fall in modes III and IV with two and three times the basic value, and there is an indication of a 5th mode with nuclei (281.7 μ^3) of 4 times the basic volume.

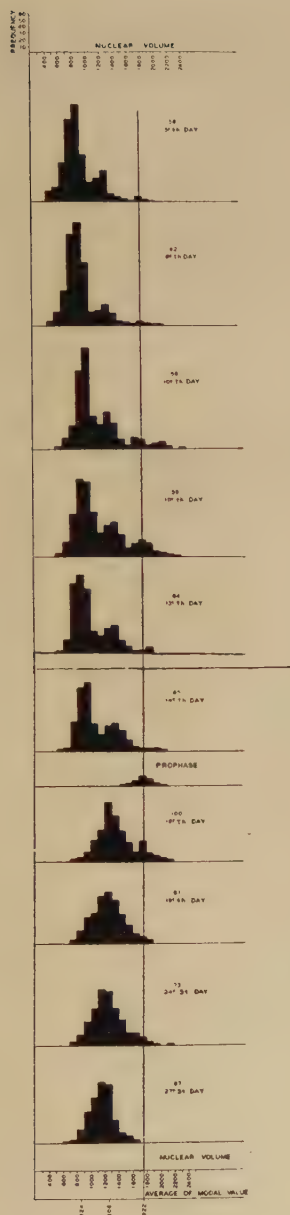


Fig. 2 Histograms of the frequency of nuclear volumes of endometrial cells during the phases of the menstrual cycle.

2. Endometrium. Proliferative phase: Histograms from the first half of the menstrual cycle (fig. 2) show three maxima of frequency with modal values of 128, 184, and $250 \mu^3$, as shown in table 3. This is a ratio of 1:1.5:2, and we may consider the volume of mode I as being basic. In all samples of the proliferative phase, and also in the two transitional specimens from days 13 and 14, the small nuclei of mode I are most numerous. The large nuclei of mode III (double the basic volume) are found mostly in samples with a high mitotic index; for example, in specimens of the 10th day, whose mitotic indices were 4.3 and 5.3% (figs. 6, 7 and 8). It is noteworthy that on the 14th day of the cycle, where mode III no longer appears, the number of mitoses is noticeably less (mitotic index of 0.6%).

Prophases (figs. 4, 7 and 8). These are significant, since they represent the end of the interphase growth of the nuclei. The small number of measurements available are grouped around mode III, indicating a nuclear volume in the prophase of double the basic size. This supports the idea that the genome is doubled in passing from mode I to mode III, in preparation for mi-

tosis, and that the transition in volume from mode II to mode III (values of 1.5 and 2 times the basic volume) must be made very quickly, the cell undergoing mitosis almost immediately after reaching the prophase.

Secretory phase: The table and histograms show, with one exception, a single modal value for nuclear volume centering around 1.5 times the basic value. Only in the 18-day sample are there a few nuclei of mode III (figs. 2, 5 and 9). Mitoses are completely lacking during the secretory phase.

DISCUSSION

We have discussed elsewhere (Salvatore, '48a, b) hypertrophy and hyperplasia in the rat myometrium under the influence of hormones or from mechanical distension by the

TABLE 3

Human endometrium

Frequencies of nuclear volumes of endometrial cells and the respective modal value in the phases of the menstrual cycle. Modal values in cubic micra and the mitotic indices are also shown

N° OF PROT.	DAY OF CYCLE	MODAL VALUE						NUCLEAR VOLUME					
		I	II	III	In cubic micra			500	600	700	800	900	1000
56	5th day	886	1273	1816	124	178	254	16	21	57	122	145	73
62	6th day	890	1295	1816	124	181	254	5	20	50	133	150	99
58	10th day	979	1307	1720	137	182	240		3	17	34	115	143
59	10th day	914	1391	1800	127	194	252		7	21	61	113	110
64	13th day	897	1383	1883	125	193	263		3	20	100	112	99
65	14th day	978	1394		136	195			1	4	43	98	100
Prophases				1807			252						
100	18th day		1309	1795		183	251				3	6	14
61	19th day			1297			181				7	19	38
73	24th day			1209			139				5	16	38
67	27th day			1206			138			3	4	20	40
Average of modal value		924	1306	1822	129	182	255						

growing embryos. We have not found mitoses in the muscle cells of the human myometrium, although Hartz ('45) has recently described them during the proliferative phase of the cycle, hence we shall consider only the question of nuclear hyperplasia during the menstrual cycle and pregnancy. Previous researches on the nuclear size of human uterine muscle cells have dealt only in linear measurements, which fail to give a true picture of growth changes going on. Table 2 gives the average measurements of a number of muscle cell nuclei in different stages of the uterine cycle, and we see that the changes in nuclear dimensions are rather slight from $17.5 \times 3 \mu$ in the proliferative phase to $19.2 \times 4.2 \mu$ in the last month of pregnancy. On converting these into nuclear volumes, however, we find that the nuclei during pregnancy average double the volume of those in the non-pregnant uterus, a significant increase.

It will be seen from the histograms of figure 1 and the data in tables 1 and 2 that the increase in nuclear volumes is not continuous but proceeds in a series of steps, with the modal points of these steps in a close approximation to the ratios 1:1.5:2:3:4. This discontinuous variation in nuclear size, with the third mode double the volume of the first or basic mode, is present during both the menstrual cycle and pregnancy. The cells of human uterine muscle thus show cycles of interphase nuclear growth quite similar to those already observed in the rat uterus.

Recent studies of intermitotic nuclear growth show a close relation between the nuclear volume and the number or size of chromosomes. Biesele, Poyner and Painter ('42), in an extensive report on nuclear volumes in neoplastic tissues, maintain the chromosomal or chromonemal nature of these discontinuous variations in nuclear volume, and emphasize that physico-chemical phenomena or water imbibition alone cannot explain why these variations occur in steps which are multiples of the basic volume, and which moreover agree with the variations in number of chromosomes or chromonemata (the

latter being determined by the number of "nucleolus organizers" and individual chromosome volume).

In like manner, we may interpret the rhythmic changes in nuclear volume which we have found in the human myometrium as showing an endomitotic nuclear growth. The term "endomitotic" is here employed in a broad sense to indicate a doubling of the nuclear genic contents, without attempting to distinguish between a separation of the multiple genomes into a polyploid number of chromosomes, or their remaining grouped in a diploid number of polytenic chromosomes. Failure of the muscle cells to undergo mitosis following upon the multiplication of the genome thus results in correspondingly larger nuclear volumes. The hypertrophy of the myometrial nuclei and cells observable during gestation is thus a genic phenomenon and not due to simple imbibition.

Endometrium. Following menstruation the epithelial cells of the endometrium proliferate under the influence of estrogen and cover the raw surface left by the sloughing of the functional layer. This multiplication of cell numbers is brought about by successive mitoses, with an interval between mitoses known as the interphase. During the interphase, although the cell must be quite active metabolically, the nucleus does not show any noticeable morphological change, other than its enlargement (interphase growth) in preparation for the next mitosis.

Our present results show that these volume variations of the endometrial nuclei are typically rhythmical. There is a group of nuclei whose volume may be considered basic, averaging $129 \mu^3$; an intermediate group averaging $182 \mu^3$; and one of $255 \mu^3$, or approximately double the basic volume. That these changes in nuclear volume are due to a reproduction of the genome is indicated by the fact that the prophase have double the basic volume, and having doubled their volume they then undergo mitosis immediately. This nuclear growth and its consequent cellular division is due to the action of estrogens, for we have observed that in spayed female rats the injection of estrone induced an interphasic nuclear growth

which doubled the nuclear volume (Salvatore, '50). In this investigation we also noted that the induction of mitosis is probably due to the drop in the level of estrogen in the blood ('48a).

An increase in nuclear volume of endometrial cells has been described by O'Leary in 1931. Analysis of his histograms shows that the two maxima, $203.3 \mu^3$ on the 2nd and $105.9 \mu^3$ on the 5th day of menstruation, approximate a ratio of 2:1. He also found an intermediate value of $161.1 \mu^3$ on the 23rd day. Although our nuclear volumes average somewhat higher (129, 182, and $255 \mu^3$), they agree with O'Leary's findings in showing a 1:1.5:2 ratio between the maxima. More recently Pfeiffer and Hooker ('44) have shown a great nuclear hypertrophy in cells of the endometrial stroma of monkeys. This hypertrophy had been induced by estradiol benzoate, and they considered it due to an increase in intracellular fluid. Neither O'Leary's nor Pfeiffer and Hooker's findings were related to cytogenetics, but they substantiate results obtained by karyometric statistics, indicating the existence of rhythmic changes in nuclear volume of genic origin.

During almost the entire second phase of the menstrual cycle (secretion phase) some condition, probably the action of progesterone, inhibits the completion of the interphase growth cycle. During this secretory phase, the nuclei show a volume about 1.5 times the basic value. This was also found in rat ovaries (Salvatore, '47b), in which cells of the Graafian follicles lose their capacity for mitotic division when changing into luteal cells. Endometrial cell nuclei also, when under the influence of progesterone, lose their ability to divide and remain stationary in the intermediate phase of intermitotic growth.

This pause in growth of human endometrial nuclei seems to be of great importance, and is related to the preparation of the endometrium for the nidation of the ovum. If implantation occurs, they probably remain stationary, or go on with their interphasic growth; on the other hand, if pregnancy does not ensue, these incomplete interphase nuclei disintegrate with the fall of hormone level and consequent menstruation.

CONCLUSION

1. Myometrium. Rhythmical variations of nuclear volume of human uterine muscle cells occur during the menstrual cycle. These variations indicate the existence of nuclear interphasic growth stages similar to those found in the oestrous cycle of the female rat. Histograms show the presence during gestation of nuclear volumes which are multiples of those found during the menstrual cycle. The average modal values in these histograms have the following ratios: 77.7:114.2:160.6:219.4 and $281.7 \mu^3$, or approximately 1:1.5:2:3 and 4. We conclude that this nuclear hypertrophy in myometrial cells during gestation is not due to simple imbibition, but is a phenomenon of genic nature, i.e., reproduction of the nuclear genome (endomitotic growth).

2. Endometrium. The endometrial growth during the proliferative phase is accompanied by nuclear interphasic growth cycles. These nuclei, which initially have an average volume of $129 \mu^3$, pass through an intermediate stage of $182 \mu^3$, and after doubling their original volume ($253 \mu^3$) undergo division.

During secretory phase, the nuclei maintain a volume approximating the intermediate stage of their interphasic growth, i.e., 1.5 times the basic volume or an average of $182 \mu^3$; and mitoses are completely absent in the endometrium.

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PLATE 1

EXPLANATION OF FIGURES

- 4 Proliferative phase of endometrium. Metaphase in one gland and prophase in the other ($5 \times$ ocul. $\times$ oil imm. $\times$ b. 35 cm).
- 5 Secretory phase of endometrium. Same magnification.
- 6 Endometrium in phase of proliferation. Note several sizes of nuclei and one mitosis ($\times 1480$).

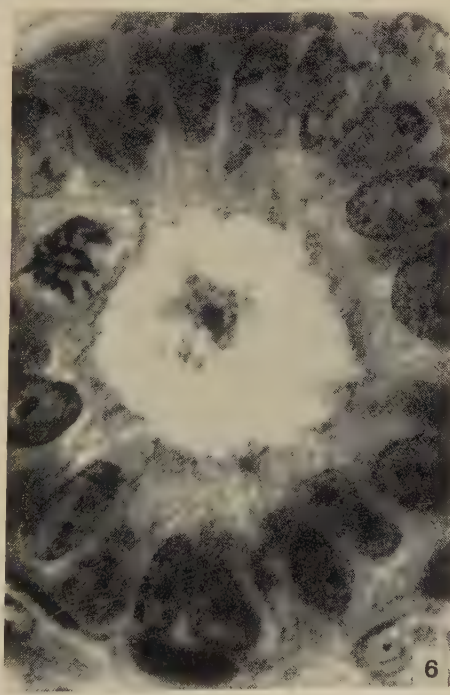
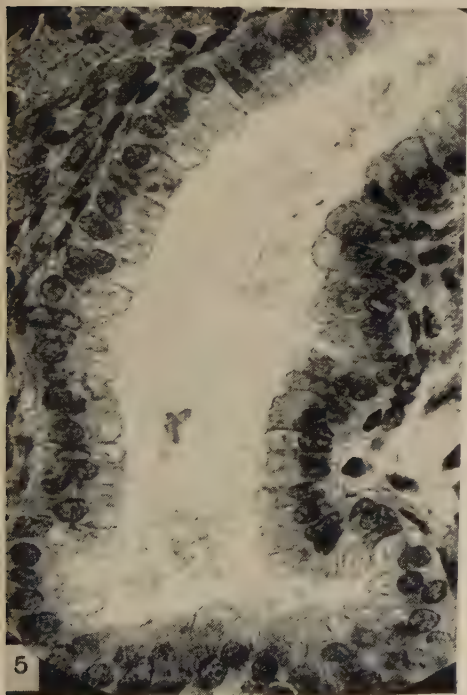
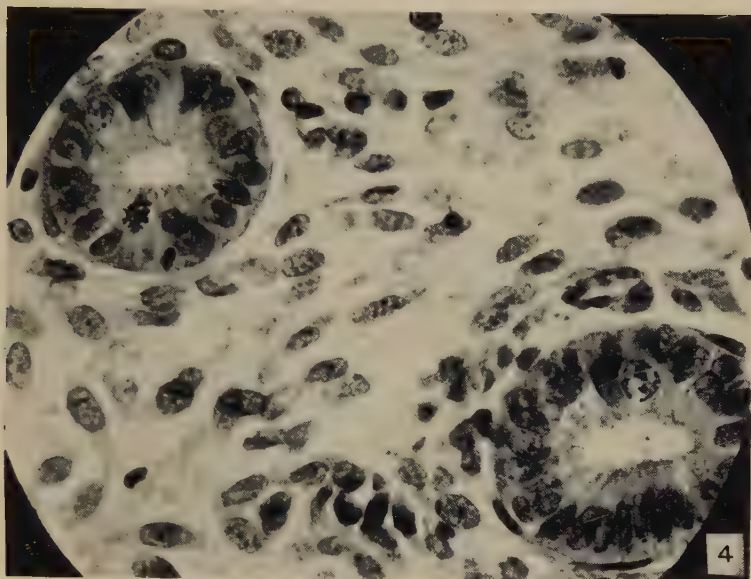
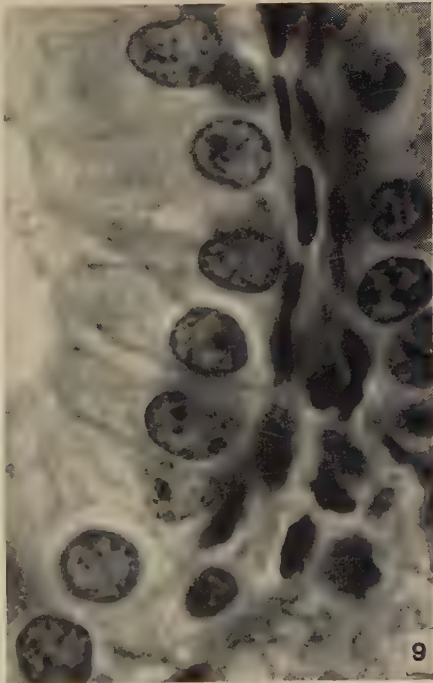
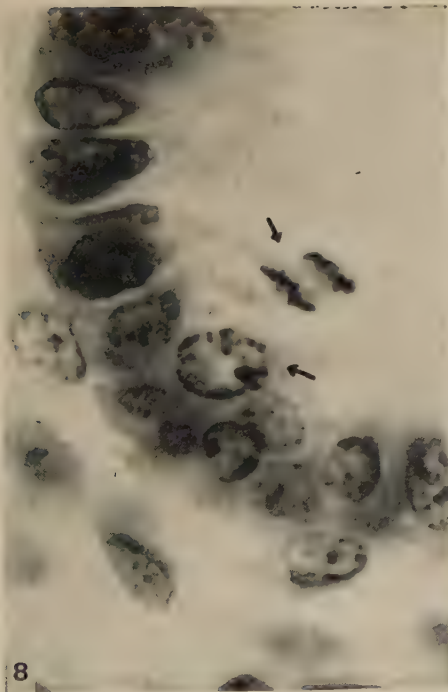
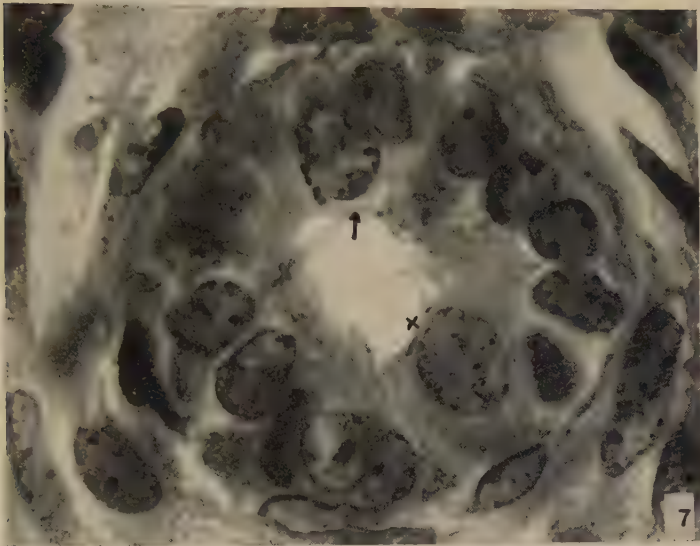


PLATE 2

EXPLANATION OF FIGURES

- 7 Same phase; notice several small nuclei, one large, and a prophase ($\times 1480$).
- 8 Showing endometrium in phase of proliferation. A typical prophase and an anaphase ($\times 1220$).
- 9 Showing secretory phase of endometrium. Notice greater regularity in nuclear sizes ($\times 1480$).



BOOK REVIEWS

Three new books have been made available for students of medicine and allied sciences who are struggling with the problem of becoming acquainted with the language of their new subjects. One is intensely practical, a new Dictionary. The other two give evidence of an increasing realization among teachers, at least in medical schools, that the direct approach, simplified spelling, and brute memory are not as effective in building a facility with language as a study of the history of words.

BLAKISTON'S NEW GOULD MEDICAL DICTIONARY, edited by HAROLD WELLINGTON JONES, NORMAND L. HOERR AND ARTHUR OSOL. 1949. xxviii + 1294 pages, 252 illustrations, 129 in color. In three bindings, \$8.50, \$10.75 and \$13.50. The Blakiston Company, Philadelphia.

Prominently placed on the jacket and the title page are the words "First Edition." This is a warning to those who have been friends of Gould Dictionaries for the last sixty years that this is not a mere revision. Gould's contribution has dwindled to the "framework." The new Editors are able men: Jones in clinical fields. Osol a specialist in pharmacology and, of particular interest to us, our own Secretary of the American Association of Anatomists, Normand Hoerr. The latter's experience as an associate editor of Gray's Anatomy and his research in neuroanatomy and cytochemistry insure a skillful coverage of the old and the new in our subject. In the preface we read, "The Editors were convinced that even the most judicious modernization of older material would prove inadequate to the need. They therefore undertook the preparation of an entirely new work —." With due modesty they realized that the task was beyond the capacity of one editor or even a small group and they called upon a staff of 80 contributors. The result indicates that they chose a group which was able not only to supply knowledge but also to cooperate in an exacting task.

A glance is enough to convince one that the book is new and modern. The vexing problem of discarding obsolete terms and uses has been adroitly handled, but the old standard eponyms, those cumbersome but pleasant relics of ancestor worship, are carefully retained. Where

else can they be found by the beginner but in a dictionary? A random sampling has shown that new words and newly expanded branches of science are competently covered.

The illustrations are brought together on consecutive pages in the central part of the book. They are accurate, quite comprehensive and scattered over a considerable range of anatomical, pathological and clinical material. Frequent reference to these illustrations are made in the text. The last 135 pages are taken up with tables of various kinds to which, again, many references are found in the text.

An interesting new feature is the treatment of pronunciation. It is possible for anyone with no training in phonetics to follow the intended pronunciation with ease and accuracy. The common rules are reviewed in a brief introductory essay, "Notes on Pronunciation." In the vocabulary, words which are difficult or do not follow the rules are respelled according to an interesting and lucid system. A particularly provocative usage in this respelling is that of a final silent "e" to designate a long vowel whether the syllable represents an actual English word or not; for example: sole, dace, dyne, style. The natural ease with which this is adopted by the reader makes one wonder whether the current tendency of orthographers to ignore semantics and drop the final "e" in words like neuron and axon is beneficial, especially when it has led to an amputation of the pleasant sounding final "e" in syndromë, turning it into an unpleasant "syndroam." Will syncope be next?

A sampling of the preference given for variants of pronunciation shows that the correct one, as established by the Merriam Webster Dictionary, is practically always favored. This is in spite of the statement in the first paragraph of the "Notes on Pronunciation" that the principle followed is to record the actual, current use and not to presume to dictate standards of correctness. A little later it is admitted that usage is not always clear-cut and the choice of variants may be arbitrary. This conservative view of the evolutionary character of language is well taken because one seldom hears *secré* tory or *baacteriós* tasis among medical people.

This is a fine book and it sets a new standard. It is possible that some may find coverage less extensive and complete for older terms than they might desire but it would be difficult indeed to criticize the accuracy or conciseness in this dictionary. The type is excellent; the use of a larger bold faced type for the entries is particularly helpful in promoting rapid and convenient use. The paper is good and the binding sturdy.

C. M. Goss

MEDICAL ETYMOLOGY, THE HISTORY AND DERIVATION OF MEDICAL TERMS FOR STUDENTS OF MEDICINE, DENTISTRY AND NURSING. By O. H. PERRY PEPPER. 1949. vii + 263 pp. \$5.00. W. B. Saunders Company, Philadelphia.

Doctor Pepper is a well known figure in medical education. He has been Professor of Medicine at the University of Pennsylvania for many years and has achieved an enviable position among his colleagues throughout the country in his speciality of internal medicine. As far as can be determined from sources at hand, his qualifications as an etymologist must be judged from this book.

The author hopes that his own interest in words and his sympathy for students will be accepted as sufficient justification for this venture. Certainly the entering student in a medical school is confronted with a difficult task. He must learn a new terminology almost at once and, with the current trend in secondary schools and colleges to slight or ignore training in language, he must find assistance somewhere. In this book the author not only succeeds in extending the helping hand by giving the derivation of words with brevity, directness and clarity, but he should kindle interest and whet the appetite with the entertaining bits of wit and anecdote which he has included.

The introduction, although it occupies but a brief three pages, gives a comprehensive view of the complex forces which have led to our modern medical terminology. In presenting the derivations, the words are grouped under subject headings and an effort has been made to place each word under the subject in which it will first be met by a medical student. The arrangement of subjects will not agree with all curricula, but it represents a commonly adopted sequence. The index is complete and can be used to track down a word not included under a particular subject where it might have been expected.

The derivations are the usual ones based on well-known authorities. In the section dealing with anatomical terms a few inaccuracies have crept in. The hyoid is called a cartilage, not a bone, shaped like the letter U. Chromaffin is seldom used to mean taking up stains, as it is given here, but ordinarily a histologist makes it refer to affinity for chromium salts. In the paragraphs introducing the section on Medicine, mention is made of the "staff of Aesculapius, the Roman god of medicine." Perhaps Homer would not admit that this is the same Asklepios who was the father of those two skilled leeches, Podalirios and Machaion, and Hippocrates might resent having it said that he assisted in the temple of a Roman god. In the history of a word, the first appearance in English is sometimes given but it is not con-

sistently pointed out when a word is of ancient lineage or whether it is a recent invention from classical roots.

In the foreword the author decries the lack of knowledge of Latin and Greek among medical students of today. Many teachers would broaden this to include workable knowledge of English. The author throws up his hands with "nothing can be done about it." We all agree with this feeling of futility as long as the Deans of our medical schools insist on pyramiding utilitarian courses in science at the expense of language, as prerequisites. It is as if they say, with Doctor Pepper, "Let us have no pedantic worrying about improper endings or cases."

This book is attractively bound, is printed on good paper in easily readable type. It should be valuable for a student but is inadequate as a reference book on etymology.

C. M. Goss

THE ORIGIN OF MEDICAL TERMS. By H. ALAN SKINNER. 1949. viii + 379 pages. \$7.00. The Williams and Wilkins Company, Baltimore.

The author is an anatomist, associate professor of Anatomy at Western Ontario, but with a medical degree and considerable interest in clinical surgery because he is a fellow of the Royal College of Surgeons of Canada.

This work is more encyclopedic than the title would lead one to expect. In addition to the etymological details there are biographical sketches and, occasionally, short essays.

The accounts of specific words are truly etymological because they contain the origin of the word, its derivation from roots and the history of its use. This is particularly valuable because it points out the difference between genuine Greek words and those coined from Greek roots to fit modern needs. The author seems to have no great fondness for these new words because many of them, especially in newer subjects like cytology, are conspicuous by their absence.

Many of the interpretations of derivation are pleasing, such as coracoid resembling a door handle rather than the usual raven's beak; mediastinum is a septum, not a space; sinus and sinusoid are generally used incorrectly. On the other hand a few derivations seem to follow conventional lines less satisfactorily. Cubitus from the Latin *cubo* to lie down because the Romans rested on their elbows when assuming a recumbent position, nothing said about eating, but this overlooks the Greek word for the ulna, kubitos, used by Hippocrates. The double "s" in masseter was used by Galen and, although it is

a corruption, it is not an English one. Muscle tone or tonus was used by Galen in his *de motu musculorum* with a meaning quite similar to the modern one. Asklepios, the hero and patron deity of medicine, is left out. One wonders whether the "followers of Aesculapius of 124 B.C." mentioned as advocating tracheotomy have not been introduced because of a confusion of Asklepiades, the Roman physician born about 124 B.C., with the ancient Greek Asklepiades who were descendants and followers of Asklepios, just mentioned. The Roman Asklepiades advocated an atomistic and mechanistic point of view and his followers founded the Methodical School of medicine.

It is unfortunate that even the best of modern scholars insist on changing the Greek ending "os" to the Latin "us" and "on" to "um." Greek origin could be recognized at a glance and much confusion avoided if the endings were not changed, especially since the alteration is used or not according to whim, for example, amnion, diencephalon, dendron, dartos; but brachium, cranium. With proper names it would be especially helpful and one could recognize the Greek association of Asklepios, Hierophilos, Erasistratos and many others by the spelling alone.

The biographical sketches form a large part of the book, taking up approximately one third of the text. In any such work it is the author's privilege to choose those whose biographies should be included. One suspects, however, that Castiglioni, for example, or a citizen of the United States or France would have made a different selection.

Much historical matter, not strictly etymological, is included. For example, the first recognition of aortic regurgitation, the discovery of the complement fixation test, and hydrogen ion concentration are commented upon. Quite extensive essays are given over to a few subjects such as the microscope, classification of animals and humoral pathology.

The bibliography at the end contains 105 references to valuable sources. There is a table of the Greek letters with their sounds and equivalents and a list of the chemical elements and their discoverers. This is a valuable source book for students and is sufficiently accurate and detailed to be used as a reference book provided the material to be looked up is among the interests of the author.

C. M. Goss

PROCEEDINGS
OF THE
AMERICAN ASSOCIATION OF ANATOMISTS
Sixty-third Annual Meeting

Notice of 1951 Meeting

THE NEXT ANNUAL MEETING
OF THE AMERICAN ASSOCIATION
OF ANATOMISTS WILL BE HELD
IN DETROIT
WEDNESDAY, THURSDAY, AND FRIDAY
MARCH 21, 22, AND 23, 1951
AT THE INVITATION OF
WAYNE UNIVERSITY
COLLEGE OF MEDICINE.

THE HEADQUARTERS HOTEL WILL
BE THE BOOK-CADILLAC.

PROCEEDINGS OF THE AMERICAN ASSOCIATION OF ANATOMISTS

SIXTY-THIRD MEETING

*Louisiana State University, School of Medicine, New Orleans,
Louisiana, April 5, 6, and 7, 1950*

*The secretary wrote a volume, more
Or less, of letters and directives beamed
To me and Dr. G., regarding lore
Of more than more anatomists, it seemed.
Association of Anatomists!*

*We hail you each and all from many nations,
Although we sometimes failed to get the gists
Of papers or to follow Pinck's gyrations.*

*Gray "G" (not goose) had mild obsessions of
Disaster and impending doom. But Low
And Ronstrom, Hamlett, Miller — all above
The norm in "go" — moved things to fast from slow.
"The sixty-third convention," wrote Herr Hoerr,
"Attained, by Randy's aid, its glorious flair."*

GEORGE WILLIAM COOPER
New Orleans
May 30, 1950

The Association held its Sixty-third Meeting in New Orleans, Louisiana, by invitation of the Louisiana State University, School of Medicine, Wednesday, April 5, Thursday, April 6, and Friday, April 7. Demonstrations and section meetings were held in the Medical School, business session at the Auditorium, Charity Hospital, general and motion picture sessions and social sessions at the Jung Hotel, the reception at the Tulane University. The Local Committee was composed of G. W. D. Hamlett, G. N. Ronstrom, F. N. Low, G. W. Cooper, R. A. Miller, and Charles M. Goss, Chairman.

Registered Attendance:

	1950	1949		1950	1949
Association Members	290	362	Out of town	438	596
Non-members	182	347	Local	34	113
	472	709		472	709

Geographical Distribution of Registrants:

Alabama	7	Minnesota	12	Texas	22
Arizona	0	Mississippi	5	Utah	4
Arkansas	6	Missouri	25	Vermont	4
California	14	Montana	0	Virginia	7
Colorado	5	Nebraska	2	Washington	4
Connecticut	6	Nevada	0	West Virginia	0
Delaware	0	New Hampshire	0	Wisconsin	17
Florida	12	New Jersey	1	Wyoming	0
Georgia	15	New Mexico	0	Dist. of Columbia	1
Idaho	0	New York	30	Belgium	1
Illinois	44	North Carolina	9	Canada	13
Indiana	10	North Dakota	1	China	2
Iowa	9	Ohio	8	England	2
Kansas	14	Oklahoma	4	France	1
Kentucky	11	Oregon	2	Germany	1
Louisiana	34	Pennsylvania	30	India	1
Maine	1	Rhode Island	2	Mexico	1
Maryland	33	South Carolina	3	Pakistan	1
Massachusetts	6	South Dakota	2	Siam	1
Michigan	25	Tennessee	9	Switzerland	1
				Uruguay	1

RÉSUMÉ OF THE SCIENTIFIC PROGRAM

The sixty-third meeting of the American Association of Anatomists was opened in general session by President Bartelmez at 10:00 A.M., April 5, in the Tulane Room of the Jung Hotel. This year President Bartelmez invited two speakers for the general session. Percival Bailey gave an address entitled *Considerations concerning the structure and function of the cerebral cortex*. Paul Ralph spoke on *The application of phase microscopy to some problems in microscopic anatomy*. The Presidential address closed the session. Dr. Bartelmez gave a review on *Variability in endocrine systems*. At the close of this session Dr. Bartelmez announced the appoint-

ment of Harold Cummins and G. W. D. Hamlett for the Auditing Committee for 1950.

The remainder of the program was divided into (a) demonstrations, Wednesday afternoon, 1:30 to 5:00; (b) a session for motion picture demonstrations, Wednesday evening beginning at 7:15; (c) sessions for the reading of papers: Thursday morning, 9:00 to 12:00; Thursday afternoon, 2:00 to 4:15; Friday morning, 9:30 to 12:00; Friday afternoon, 1:30 to 3:30; (d) the business session, Thursday at 12:15. On the Thursday morning, Thursday afternoon, Friday morning and Friday afternoon sessions there were 5 concurrent programs.

NUMBER OF PAPERS AND DEMONSTRATIONS

	1950	1949	1948
Papers from platform	195	233	201
Papers withdrawn	10	5	5
Papers actually presented	185	228	196
Papers read by title	72	78	72
Demonstrations	52	66	45
Motion pictures	7	14	16

SOCIAL ACTIVITIES

Banquet: Attendance (314). Tulane Room, Jung Hotel. After-dinner speaker: Dr. Herbert M. Evans, University of California, Berkeley, California, who spoke on "Some Unsolved Problems in Anterior Pituitary Physiology."

Smoker: Approximately 500. Tulane Room, Jung Hotel.

Informal Reception: Attendance (450). Tulane University, Rudolph Matas Medical Library, Hutchinson Building (adjoining Charity Hospital).

The New Orleans meeting was particularly notable in the large number of wives who accompanied their husbands. Their attendance added greatly to the enjoyment of the social events.

MINUTES OF THE FIRST BUSINESS SESSION

April 6, 1950, 12:15 P.M.

President Bartelmez announced the appointment of the Nominating Committee for 1951: H. Stanley Bennett, James O. Foley, and Davenport Hooker, Chairman.

The President reported that the minutes of the Sixty-second Session were printed in The Anatomical Record, v. 106, no. 2, February, 1950, reprints of which had been distributed to all members, and would not be read unless called for. On motion duly made and seconded, the minutes were approved as printed.

Report of the Treasurer: The Secretary-Treasurer made the following report for the year 1949:

Financial Statement for the Year 1949

Balance on hand in checking account, December 31, 1948, in	
The Cleveland Trust Company, Cleveland, Ohio	\$2,310.24
Receipts from dues	2,637.30
Sale of Abstracts and Proceedings	117.66
Total Credits	\$5,065.20
<i>Expenditures</i>	
Postage, supplies and miscellaneous	86.04
Wistar Institute, Philadelphia, Pa.	
Preliminary notices	313.81
Abstracts	676.87
Programs	508.82
Addressograph additions and corrections	11.24
Printing	3.60
Philadelphia Meeting Expense	600.00
(Paid to Local Committee)	
Enrollment	
National Society Medical Research	100.00
National Research Council Dues A.I.B.S.	659.00
Total Expenditures	2,959.38
	\$2,105.82
Balance on hand December 31, 1949, and deposited in the	
name of The American Association of Anatomists in The	
Cleveland Trust Company, Cleveland, Ohio	\$2,105.82
War Bond Defense Series F dated May 1942 in safe deposit	
box, Central National Bank, Cleveland, Ohio	
Value December 31, 1949	848.00
Total Assets	\$2,953.83

ANATOMICAL JOURNAL TRUST FUND

1949

Bonds on hand December 31, 1949		\$17,000.00
Cash on hand Central National Bank, Cleveland, Ohio, December 31, 1948	629.30	
Interest received from bonds during 1949	225.00	854.30
Total Assets		\$17,854.30

No expenditures were made from this fund during 1949.

Report of the Auditing Committee: Dr. Cummins read the following report:

"The undersigned committee has examined the accounts of the Secretary-Treasurer for the year 1949 and has found total receipts to be those entered in the journal, expenditures in accord with receipted bills, and the balance on hand December 31, 1949, in agreement with the bank statement of that date."

(Signed) HAROLD CUMMINS
GEORGE W. D. HAMLETT

On motion made and seconded the reports of the Treasurer and of the Auditing Committee were accepted and adopted.

Election of Officers: The Nominating Committee for 1950 consisting of W. E. Sullivan, Chairman, Horace W. Magoun, William U. Gardner, Allan L. Grafflin, William W. Greulich, placed the following nominations before the Association: Sam Clark, Vanderbilt University School of Medicine, for President for a two-year term; Gordon Scott, Wayne University College of Medicine, for First Vice-President for a two-year term; J. C. B. Grant, University of Toronto, for Second Vice-President for a two-year term; Normand L. Hoerr, Western Reserve University School of Medicine, for Secretary-Treasurer for a four-year term; Donald Duncan, University of Texas, and Miriam Simpson, University of California for election to the Executive Committee for a four-year term; Louis B. Flexner, Carnegie Institution, to serve 3 years of Dr. Scott's unexpired term, if he is elected First Vice-President.

In the absence of additional nominations a motion was made, seconded and carried that the Secretary cast a unanimous

ballot for the above-named nominees, and they were declared elected.

Election of Representatives and Committee Members: The Secretary presented, in behalf of the Executive Committee, the following appointments: Representatives to the meeting of the American Association for the Advancement of Science in Cleveland, 1950, R. A. Knouff and Normand L. Hoerr.

The Executive Committee appointed a special committee to study the question of establishing an anatomical review journal, the committee to consist of Drs. Harold Cummins, Chairman, Warren O. Nelson, and Ernst Scharrer. This committee is to report at the next meeting.

Membership report: The Secretary presented in brief the following report:

On March 1, 1950, the Association had 906 members. This number has been reduced during the year by 6 deaths and 4 resignations. (At present, 43 members are in arrears in payment of dues ranging from one to three years.)

Members deceased: The Association has suffered very great loss through the recent deaths of the following 6 members: Esther L. Boyer, William Darrach, William Washington Graves, Norman W. Ingalls, Augustus G. Pohlman, and Adolf Meyer.

We have received the following from Dr. Philip E. Smith, College of Physicians and Surgeons, Columbia University.

DR. WILLIAM DARRACH died on May 24, 1948, at the age of seventy-two. During his medical course he spent all available hours working with the late Dr. George Huntington. Following his internship he was Demonstrator in Anatomy from 1908-1909 at the College of Physicians and Surgeons, Columbia University. He served as Dean from 1919-1930. During World War I he rendered conspicuous service in the army medical corps and in World War II spent many hours in the dissecting room instructing groups of army medical officers. Dr. Darrach retained his love of anatomy throughout his busy career.

The following was received from the Reverend A. M. Schwitalla, the former Dean of St. Louis University School of Medicine.

DR. WILLIAM WASHINGTON GRAVES was one of the physicians who bridged over the changes in neuropsychiatry from the last two decades of the previous and the first half of the present century. Born in 1865 in LaGrange, Kentucky, and graduated with a Doctor of Medicine degree from the St. Louis College of Physicians and Surgeons in 1888, he early turned his special attention to the study of the basic sciences of anatomy, pathology and anatomic neurology, thus laying the ground work for his future specialization. He spent almost three years in Europe, particularly in Germany and Austria, as a roaming student, visiting all the great universities and registering for special courses under the great masters, the outstanding teachers of the first decade of the present century. When he returned to St. Louis in 1905, he became instructor in the department of Neurology in the St. Louis University School of Medicine and continued as a faculty member of that institution up to the year 1948 when he became Emeritus Professor and Director Emeritus of his department. He was appointed to the professorship in 1914 and to the directorship of the department of Neurology and Psychiatry in 1925. He served as consultant in neurology in practically all of the hospitals of St. Louis at one time or another, both in the public as well as in the private institutions.

Dr. Graves' investigations, it is interesting to note, dealt with problems that were at first sight quite remote from the area of his clinical practice. The study of the structure and the characteristics of the shoulder blade might well at first sight seem to be a purely morphological study; for Dr. Graves, however, that study was the beginning of a long pathway into the unknown which he had projected and on which he progressed during more than 30 years, not very far, it must be admitted, but sufficiently far to have his efforts achieve significance. Dr. Graves became more and more interested in the relation between physical and mental characteristics and

while it must be confessed that research in that field progressed faster than Dr. Graves himself could follow, nevertheless, his own personal philosophy won for him an invitation to discuss his viewpoints before the University of Edinburgh. If Dr. Graves' morphological studies had been conducted a generation earlier, they would, no doubt, have contributed greatly to the progress of morphological and statistical genetics. As it was, they were conducted during the time when the study of heredity was leading into physiological and biochemical interpretations. Dr. Graves sensed this and with his accustomed insistent determination, he attempted to follow. Unfortunately, his health began to fail fully 15 years ago, forcing him to place a limit on the incredibly long hours of devotion to the study which he had determined to make his life work. His mass of data and his osteological collection represent an expenditure of energy and time equalled to the knowledge of the writer by no other investigators even by those who gave their full time to their research.

To know and understand Dr. Graves, one must understand and know not only his scientific and his clinical work but also his human characteristics, his kindliness, his broad sympathies, his desire to alleviate suffering — a desire which had been enormously stimulated by reason of his own personal sufferings, his idealism and above all, his ability to interpret favorably to the individual almost any kind of transgression. He was one of those rare individuals who applied in his own life, the full truth of the proverb, "To know all is to excuse all." By nature and by choice, innately as well as by his education and by his self-discipline, he was a gentleman. His long period of failing health and his sufferings were terminated by death on April 18, 1949.

Professor L. E. Noland, Department of Zoology, University of Wisconsin, submitted the following biography.

ESTHER LYDIA BOYER was born at Reddick, Illinois, on March 27, 1902. She took her B.A. degree at Western Union College in 1923, and became an assistant in zoology at the University of Wisconsin in 1929. She received her M.A. degree

there in 1930. Her work for the doctorate was done in the Anatomy Department of the same university on some comparative aspects of primate and human anatomy. She served as an assistant in anatomy from 1934 to 1939. After receiving her Ph.D. degree at Wisconsin in 1933, she entered the Medical School there, and was granted the M.D. degree in 1939. Following this she spent a year of internship at the Madison General Hospital in Madison, Wisconsin, in 1939-40. She then accepted an instructorship in anatomy at Woman's Medical College in Philadelphia. After two years in this position she became assistant physician in the student health service of the University of Missouri at Columbia. In 1944 she left Missouri to become a member of the Western Montana Clinic in Missoula, where she served for 4 years as physician and surgeon. While there she published with Dr. H. M. Blegen a paper on perforation of choledochus cyst with biliary peritonitis (*Lancet*, 66: 177, 1946). On February 7, 1948 she fell while walking in the mountains alone, and lay so long unconscious that one foot was frozen. About a month later (March 5, 1948) when she appeared to be recovering nicely from this accident she died quite suddenly. She was buried near her birthplace in Reddick, Illinois. She was a gentle, unselfish person, persevering in the tasks she set for herself, devoted to her work, constant and genuine in her friendship, and interested always in adding to the health and happiness of mankind.

The biography reproduced herewith was submitted by Dr. Samuel L. Chase, Department of Anatomy, Western Reserve University.

NORMAN W. INGALLS was born in LaGrange, Ohio, August 10, 1880, the son of Miles W. and Laura Johnson Ingalls. He received a B.S. degree from Baldwin (now Baldwin-Wallace) University in 1901, an M.D. from Western Reserve University in 1905, when he became Demonstrator of Anatomy in the School of Medicine. He served as Instructor from 1906 to 1909, Assistant Professor from 1909 to 1913, and Associate Professor from 1913 until his retirement because of ill health in 1937. In 1906-1907 he studied at Leipzig and Heidelberg,

Germany, in 1913-1914 held a Fellowship at Victoria College, Manchester, England. He also studied at Johns Hopkins University. Among other organizations he was a member of the American Association of Anatomists, the American Association for the Advancement of Science, Phi Rho Sigma medical fraternity, Alpha Omega Alpha and Sigma Xi honorary societies. He died July 27, 1949, survived by his widow, Dorothy Butts Ingalls; a son, William L.; a daughter, Mrs. Virginia Hathaway; a sister, Flora Ingalls; and two grandchildren.

Doctor Ingalls' primary and continuing interest was embryology, as shown by the fact that 18 of his 31 scientific reports were on embryological and teratological subjects. A secondary interest, from association with T. Wingate Todd, led to the publication of 9 papers in the field of anthropometry and three in ethnology. He was a true scholar whose encyclopedic knowledge was a never failing, graciously shared source of aid and inspiration to colleagues and students.

DR. AUGUSTUS G. POHLMAN was born in Buffalo, New York, on February 21, 1879, and received the M.D. degree from the University of Buffalo in 1900. He taught anatomy at Cornell, Johns Hopkins, Indiana, St. Louis University, and Creighton. In his later years he was associate professor of otolaryngology at the University of Southern California. He died in Los Angeles on April 1, 1950. Most of his research work concerned the comparative anatomy, embryology, and physiology of the ear, in which fields he made notable contributions.

DR. ADOLF MEYER'S biography is being published separately.

Members resigned: Ruggles K. George, B. E. Hall, Richard G. Abell, Caroline McGill.

The resignations were accepted with regret.

Members dropped for non-payment of dues for two years or more: The Secretary announced that the following member had been dropped for non-payment of dues by action of the Executive Committee. This member has been in arrears for 4 years.

James B. Looper

(The Constitution, in Article VI, Section I, provides that former members "may be reinstated at the discretion of the Executive Committee on payment of arrears.")

Election of New Members: The Secretary presented the following 33 names of persons elected by the Executive Committee to membership in the Association, the names in parentheses indicating the sponsors.

- KENNETH R. BRIZZEE, B.S., U. of Utah, 1939; M.S., U. of Utah, 1941; Ph.D., St. Louis University, 1949; Instructor in Anatomy, University of Utah College of Medicine. (T. F. Dougherty, E. Hashimoto.)
- Tso KAN CHANG, Ph.D., University of Connecticut, 1949; Teaching fellow, Department of Anatomy, School of Medicine, Western Reserve University. (J. S. Nicholas, A. Lazarow.)
- MAURICE CHEVREMONT, M.D., University of Liège, 1934; "Agrégé de L'Enseignement supérieur," University of Liège, 1942; Professor of Histology and Embryology, University of Liège (Belgium). (K. Porter, N. L. Hoerr.)
- CLARENCE MURPHY COMBS, Ph.D., 1950 (presumptive), Northwestern University; B.A., Transylvania College, 1946; M.S., Northwestern University, 1948; Ward Fellow in Anatomy, Northwestern University Medical School. (W. J. S. Krieg, H. W. Magoun.)
- JAMES N. DENT, A.B., University of Tennessee, 1938; Ph.D., Johns Hopkins University, 1941; Associate Professor (Acting) Biology, University of Virginia. (J. E. Kindred, C. C. Speidel.)
- WILLIAM RANKIN DURYEE, Ph.D., Yale, 1933; A.B., Yale, 1927; Staff Associate (Cytologist), Carnegie Institution of Washington, National Cancer Institute. (C. C. Speidel, J. E. Kindred.)
- LAURENCE ROCKWELL FITZGERALD, Ph.D., Iowa, 1949; B.S. Tufts College, 1939; M.S., Iowa, 1941; Instructor in Anatomy, University of Tennessee. (F. Harrison, R. H. Alden.)
- ROBERT GETTY, B.V.M., Ohio State University, 1940; M.S., 1945; Ph.D., 1949, Iowa State College; Associate Professor, Iowa State College. (H. L. Foust and N. L. Hoerr.)
- LOIS A. GILLILAN, M.D., Assistant Professor of Anatomy, University of Pennsylvania, School of Medicine. Reactivation of membership.
- JOHN CHAMBLEES HALEY, M.D., Baylor University College of Medicine, 1927; B.S., East Texas State Teachers College, 1929; Professor of Anatomy and Chairman of Department, Baylor University College of Medicine. (G. G. Robertson, I. R. Telford.)
- WALTER FEARN HARPER, B.Sc., 1923, Ph.D., 1927, M.B., Ch.B., 1931, M.D., 1934, University of St. Andrews; Professor of Anatomy, University College of the West Indies, Mona, Jamaica. (J. D. Boyd, W. G. Hamilton.)
- ROBERT A. KAISER, A.B., Niagara University, 1938; M.D., University of Buffalo, 1942; Instructor in Anatomy, School of Medicine, University of Buffalo. (O. P. Jones and G. L. Rasmussen.)

- MARGARET A. KELSALL, A.B., 1939, M.A., 1940, Ph.D., 1943, University of Colorado; Research associate, Department of Biology, University of Colorado. (E. M. Vicari, G. J. Noback.)
- ELIZABETH HORTENSE LEDUC, Ph.D., Brown University, 1948; B.S., University of Vermont, 1943; M.A., Wellesley College, 1945; Instructor in Anatomy, Harvard Medical School. (G. B. Wislocki, E. W. Dempsey.)
- CHAN-NAO LIU, Ph.D., University of Pennsylvania, School of Medicine, 1949; B.S., Peiping National Normal University, China, 1935; Instructor in Anatomy, University of Pennsylvania, School of Medicine. (W. F. Windle, R. G. Williams.)
- EDWARD M. NELSON, B.A., 1937, M.A., 1939, Ph.D., 1947, University of Wisconsin; Associate in Anatomy, Stritch School of Medicine, Loyola University. (T. T. Job, A. J. Gatz.)
- WILLIAM T. NIEMER, M.Sc., 1938, University of Cincinnati; Ph.D., 1946, Cornell University; Instructor in Anatomy, Northwestern University Medical School. (H. W. Magoun, A. R. Vonderahe.)
- GEORGE EMIL PALADE, M.D., Faculty of Medicine, University of Bucharest, 1940; Fellow at the Rockefeller Institute for Medical Research. (K. R. Porter, N. L. Hoerr.)
- JEROME PATRICK PARNELL, Ph.D., New York University, 1949; B.S., Manhattan College, 1941; M.S., St. Louis University, 1943; Assistant Professor, Long Island College of Medicine. (J. B. Hamilton, H. Cummins.)
- JOHN W. PATTERSON, M.D., Western Reserve University, 1949; A.B., Ohio Wesleyan University, 1939; M.S., 1941, Ph.D., 1942, Ohio State University; Assistant Professor of Anatomy, School of Medicine, Western Reserve University. (N. L. Hoerr, A. Lazarow.)
- IRVING REHMAN, B.Sc., 1933, M.S., 1935, Ph.D., 1939, New York University; Associate Professor of Anatomy, University of Southern California School of Medicine. (P. Patek, N. L. Hoerr.)
- RANDALL WILLIAM REYER, B.A., Cornell University, 1939; M.A., Cornell University, 1942; Ph.D., Yale University, 1947; Instructor in Zoology, Yale University. (L. S. Stone, J. S. Nicholas.)
- GEORGE JOHN ROMANES, B.A., Cantab., 1938; Ph.D., Cantab., 1941; M.B., Ch.B., Edinburgh, 1944; Lecturer in Neuroanatomy, University of Edinburgh, Scotland; at present Commonwealth fellow, Department of Neurology, Columbia University. (F. A. Mettler, G. J. Noback.)
- JERZY EDWIN ROSE, M.D., Jagiellon University, Gracov, Poland, 1937; Assistant Professor of Physiology and Psychiatry, Johns Hopkins University. (W. L. Straus, F. N. Low.)
- KATHERINE KOONTZ SANFORD, B.A., Wellesley College, 1937; M.A., 1939, Ph.D., 1942, Brown University; Cytologist, National Cancer Institute, Bethesda, Maryland. (W. R. Earle, G. H. Algire.)
- ROGER WOLCOTT SPERRY, Ph.D., University of Chicago, 1941; A.B., Oberlin College, 1935; A.M., University of Chicago, 1937; Assistant Professor of Anatomy, University of Chicago. (G. W. Bartelmez, S. Polyak.)
- FRITZ STRAUSS, M.D., University of Freiburg i. Br., 1932; Privatdocent and Professor, Department of Anatomy, University of Berne, Switzerland. (H. W. Mossman, W. Sullivan.)

- SYDNEY SUNDERLAND, B.S., M.B., 1935, D.Sc., 1945, M.D., 1946, University of Melbourne; Professor of Anatomy and Histology, University of Melbourne, Australia. (H. W. Magoun, N. L. Hoerr.)
- YU-CHUAN TSANG, B.A., National University of Peking, 1925; Ph.D., University of Chicago, 1934; Professor of Anatomy, The Medical College, Peking National University, China. (G. W. Bartelmez, N. L. Hoerr.)
- CHARLES MERREL WALDO, B.A., University of Michigan, 1928; D.D.S., 1930; M.S., 1932; Associate Professor of Orthodontics, Harvard School of Dental Medicine. (G. B. Wislocki, E. W. Dempsey.)
- RAY L. WATTERSON, B.A., Coe College, 1936; Ph.D., University of Rochester, 1941, Associate Professor of Biology, Northwestern University. (B. H. Willier, M. E. Rawles.)
- JU-KANG WOO, Undergraduate work taken at National Central University of Nanking, China; M.S., 1948, Ph.D., 1949, Washington University; Teaching Human Anatomy, Dairen University School of Medicine, Dairen, China. (Mildred Trotter, E. V. Cowdry.)
- GEORGE WALTER WOOLLEY, B.S., Iowa State College, 1930; M.S., 1931, Ph.D., 1935, University of Wisconsin; Head of Division of Steroid Biology, Sloan-Kettering Institute, Memorial Hospital Center. (E. M. Vicari, G. J. Noback.)

On motion duly seconded and passed unanimously the election of these new members was ratified.

Members placed in the category of "members relieved of the payment of dues": The Secretary reported, that by action of the Executive Committee, 8 members had been placed in this category in accordance with the provisions of Article VI, Section II, of the Constitution, which reads: "Any member who has paid the annual dues for 30 years, or who has attained the age of 65 years, or who has retired because of illness, may be relieved of the annual dues at the discretion of the Executive Committee." It was stated that this brings to 52 the total number in this category.

Number of members:

Total membership April 14, 1949	906
Deceased during year	6
Resigned during year	4
Dropped for non-payment of dues	1
	—
	895
New members elected April 4, 1950	33
	—
Total membership April 4, 1950	928

Payment to Local Committee: The Secretary presented the recommendation of the Executive Committee that the Local Committee be allowed \$600.00 to defray costs of the meeting. On motion duly made and seconded it was voted that the Treasurer be authorized to pay the sum of \$600.00 from the general funds of the Association to the Local Committee.

Place of next meeting: It was announced on behalf of the Executive Committee that the Committee had voted to accept an invitation from Wayne University, Detroit, to meet there in 1951, March 21, 22, and 23. The headquarters hotel will be the Book-Cadillac.

Appropriation of \$100 to the National Society for Medical Research: It was again voted to pay the National Society for Medical Research \$100 in recognition of the splendid work of this society.

The American Institute of Biological Sciences: As our representative to the governing board of the A.I.B.S., Dr. Louis Flexner read the following report on the activities of this society for the past year:

Member Societies. The Institute now has 15 full member societies and two affiliate societies.

Officers. E. G. Butler, Chairman; F. P. Cullinan, Vice Chairman; Milton O. Lee, Executive Secretary.

Financial Report

Estimated Income	\$9,200
Estimated Expenses	
Salaries	\$6,200
Addressograph System ..	600
Travel Expenses	1,900
Governing Board	1,200
Staff	700
Office Expenses	500
Total Expenses	\$9,200

The budget is based entirely upon income from societies. The Executive Committee is now laying careful plans for a campaign to enlist industries as associates.

Specific activities of the Institute include:

Handbook of Biological Data. In March 1949 a contract was negotiated with the Air Material Command for the compilation of a Handbook of Biological Data, analogous to the existing handbooks of chemistry and physics. A committee was constituted consisting of the following:

Theodore Byerly, Chairman; Harold J. Conn; David R. Goddard; Charles M. Goss; William W. Greulich; F. G. Hall; J. W. Heim; Paul E. Howe; E. Morton Jellinek; Adolph H. Schultz; Paul Weiss; and Errett C. Albritton, Executive Secretary and Editor.

The Handbook, when completed, will cover all fields of biology and biological data which are either quantitative and may be expressed in numerical tabular form or which may be reduced to concise tabular form, though not numerical.

Committee on Selective Service. A Committee on Selective Service was appointed consisting of J. S. Nicholas, E. G. Butler, L. J. Stadler and Leland W. Parr, Chairman. This committee was chosen by General Hershey as his advisory panel in the field of biology and took the lead in the development of a plan for educational deferment which treats students in all fields on an equal basis. This plan has been officially approved and is ready for adoption in case Selective Service is revived.

Committee Advisory to the Armed Services. At the request of the General Staff, U. S. Army, there has been set up in the A.I.B.S. a committee which has as its function the review of such questions as the Armed Services may wish to propose to it, particularly those concerned with the acquisition of information concerning the locations and numbers of qualified individuals who could serve in scientific capacities in either general or specific categories, and to act in general upon those problems of the Armed Services which specifically demand competent scientific review. Dr. J. S. Nicholas is chairman of the committee and the members are Wallace O. Fenn, Norman H. Giles, Jr., Ira V. Hiscock, Harold B. Tukey, LeRoy Voris, and Herman S. Wigodsky.

Committee on Publications. The activity of the committee has been centered around improved publication procedures and around the possibility of a central business and editorial office.

As reported earlier, the editors and business managers of the biological journals have been canvassed to determine their interest in improving publication procedures. Some were not interested, many were moderately interested, and many others were greatly interested.

Consulting Service for the Office of Naval Research. At the request of the Chief of Naval Research, the A.I.B.S. has entered into the negotiation of a contract providing for a consulting service for the Office of Naval Research in the field of biology. The function of A.I.B.S. through committees of experts will be to advise on the initiation and

continuation of projects, to suggest new research projects and to keep informed on the progress of active projects. Funds to meet the cost of these services will be provided by the O.N.R.

Survey of Biological Research Potential in Universities, Colleges and Non-Profit Institutions. The A.I.B.S. has initiated a survey to determine the nation's research programs in this field. The Bureau of the Budget has requested such information from the Institute for the purpose of estimating the amount to be allocated to biology by that office in the event of the establishment of a National Science Foundation. Such information is also important in connection with the allocation to the biological sciences of presently available funds from existing Governmental agencies and other sources.

Meetings. At the request of the secretaries of biological societies meeting in Washington, September 1948, the Institute has worked closely with the A.A.A.S. in developing plans for the New York Meetings. The Institute was asked by various member societies to look into the possibility of organizing a meeting for societies for 1950 and has made arrangements to hold such a meeting on the campus of Ohio State University, September 11 through 13, 1950.

Addressograph Plates. Addressograph plates of the entire membership will be finished by January 1, 1950 and the first issue of the 1950 NEWS LETTER will be sent to this list.

The following charges for the use of the plates were approved by the Executive Committee:

Member societies will be allowed to use the plates without charge; associates and affiliates will be allowed to have one complete list without charge per year and subsequent lists will be \$10.00 per thousand addresses; universities, presses, etc., will be charged two cents per address.

The NEWS LETTER will contain much information of general interest to biologists and will be sent to heads of biological departments as well as to A.I.B.S. members.

National Salary Survey of Biologists. The Office of Scientific Personnel of the National Research Council is planning to make a survey of the economic status of all scientists under contract with a Government agency and has asked the A.I.B.S. to assist in the field of biology. The purpose of such a survey is to inform Congress as to the comparative salaries of industrial, academic and Government research scientists.

Inter-Society Color Standards. At the request of the Inter-Society Color Council, the A.I.B.S. conducted a survey among biological departments to determine their need and demand for more complete

color standards. Results to date indicate that about 90% of the returns are in favor of the production of more adequate color standards for the field of biology.

Report of M. O. Lee, Executive Secretary
American Institute of Biological Sciences
as condensed by L. B. Flexner

It was voted to renew our membership in the A.I.B.S. for another year.

Dues for 1950: The Secretary-Treasurer reported that the Executive Committee had recommended that the dues for the year 1950 be raised to \$5.00. This is \$1.50 above the \$3.50 level which has existed in 1949. After considerable discussion, this recommendation was voted.

Report of the Motion Picture Committee: Dr. Carl Speidel read the following report.

The Committee repeats, in part, a statement reported in the 1949 Proceedings:

“In 1940 The Wistar Institute of Anatomy and Biology, under Dr. E. J. Farris, created, at the The Wistar Institute, a depository for motion picture films of anatomical and zoological interest. The scheme has provided a central depository and clearing-house for biological motion picture films of both research and teaching interest that have not yet been submitted for review. This Committee suggests that producers who possess such reels, and would like to have them available to others, send them to Dr. E. J. Farris, The Wistar Institute, Woodland Ave. and 36th St., Philadelphia 4, Pa., for consideration and, if approved, for listing, with other approved motion picture films, in the Anatomical Record. For style of listing consult the Anatomical Record, Vol. 101, at page 132, 1948 (which also gives a list of approved films). For details write to Dr. Farris.”

While the main purpose of the original plan was to provide a central location where films would be available to *research* workers, in practice it has been found that many of the listed films are also of value and have been widely used in the *teaching* of Anatomy. In response to requests of members of

the Association of Anatomists, Dr. Farris will gladly list *available* films that are chiefly of teaching interest, either as films endorsed by The Wistar Institute, or, in supplementary categories, as (1) films endorsed by the Motion Picture Committee of the Anatomists, or as (2) films reported to — or known by — the Committee to be useful teaching films. In any category the Wistar style of listing should be followed as fully as possible. Also, The Wistar Institute would appreciate, as in the past, the deposition with them of a copy of the film, although this is not required.

If this proposal meets with the approval of the Association, it is urged that members not only submit films for review, as urged above, but that they also send to Dr. Farris information regarding films — *available* for loan, rent or sale — which they have found useful in teaching. It is suggested that the lists, when published, be included either as part of, or along with the Proceedings of the Meetings of the Anatomists, in order that each member may automatically receive a copy.

(Signed) ELIOT R. CLARK
for the Committee

The American Journal of Anatomy: The Secretary reported that the Executive Committee had approved Dr. Cummins' appointment of C. P. LeBlond as associate editor of the Journal, to take the place of Dr. Joseph Hinsey, who had resigned.

National Research Council: Dr. Marion Hines, our representative, gave a most illuminating and edifying report of the last meeting of the Council. No business of interest to our Association was considered.

Dr. Louis Flexner made the following motion:

"Resolved: The American Association of Anatomists opposes any provision for FBI investigation and clearance in non-secret fields of investigation sponsored by a National Science Foundation.

"The Association agrees with the House-Senate Conference proposal that FBI investigation and National Science Foundation clearance be required for all persons having access to matters involving national security."

The motion was voted unanimously.

Dr. Gordon Scott moved a vote of thanks to the local committee for all of their hard work and hospitality, which made this meeting such a great success.

This motion was passed unanimously.

Thanks were also voted to Tulane University for their hospitality at the Reception.

The meeting adjourned at 1:05 P.M.

THE CAJAL CLUB

A report from Dr. Pinckney Harman is reproduced in part herewith.

“The Cajal Club was originated during the Montreal Meetings of the American Association of Anatomists in 1947 by a group of anatomists who had been brought into close association with one another through a common interest in the nervous system. During subsequent Meetings a program of activity has been formulated. The Club is supplementary to the Association of Anatomists, and its scientific program is designed to foster discussions which for one reason or another are not suitable for presentation at the Annual Meetings.

The Club has now advanced to the point that we may look forward to yearly foregatherings on the day preceding our Annual Meetings. The schedule as planned includes papers which could not be presented later; e.g., those dealing with history of neurology, long reviews of special interest of individual members, prospective research, technique, and discussions on the teaching of neuroanatomy.

The Cajal Club held its 4th annual meeting in conjunction with the general meeting of the American Association of Anatomists at 10:00 A.M., Tuesday, April 4, 1950, in Room 701 of the Louisiana State University School of Medicine, New Orleans, Louisiana.

Papers were presented as follows:

1. Some theoretical considerations of stereotaxic machines. WENDELL J. S. KRIEG, Department of Anatomy, Northwestern Medical School.

2. Use of domestic cresyl echt violet as a neurological stain. GEORGE CLARK and THERESA M. BANNY, Department of Anatomy, Chicago Medical School.

3. Some recent experiences with Golgi method. CLEMENT A. FOX, Department of Anatomy, Marquette University School of Medicine.

4. Experimental studies on the origin of the autonomic ganglia of the chick embryo. DAVID S. JONES, Loyola University School of Medicine.

5. Cajal's contribution to corticoarchitectonics. A. VAZ FERREIRA, Department of Anatomy, Northwestern Medical School.

6. Staining of paraffin sections with silver chloride. GEORGE J. ROMANES, Department of Neurology, Columbia University.

Presentation of papers was followed by a business session, and the day's activities were concluded with an intermission at Pat O'Brien's and a banquet at Arnaud's.

(Signed) PINCKNEY J. HARMAN
Apical Dendrite

AMERICAN ASSOCIATION OF ANATOMISTS

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